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EFFECT OF DILUTION ON THE pH OF SOILS TREATED WITH VARIOUS CATIONS

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There has been much discussion as to the ratio of soil water that should be used in determining hydrogen-ion concentrations. In a recent publication (11) the International Society of Soil Science recommends for experimental work a soil water ratio of 1:2.5. For mineral soils Snyder (13) recommends a ratio of 1:2. Billmann and Jensen (3) in working with Danish mineral soils used a ratio of 1:1. Barnette, Hissink, and Van Der Spek (2) recommend the thicker suspensions as showing a more constant hydrogen-ion concentration. There are many other publications on this subject and most of them recommend a low soil-water ratio. Snyder (13) has satisfactorily reviewed the literature on this subject. The consensus is that small amounts of water are to be desired.

THE PROBLEM

The authors of the present paper, bearing in mind the base-exchange and electrodialysis work of Gedroiz (5), Hissink (8), Kelley (9), Mattson (10), and Bradfield (4), decided to determine the effect of dilution upon the reactions of soils when they contained various cations. The basic ions selected for use were those more commonly found in soils and were H^+, NH_4^+, Na^+, K^+, Ca^{++}, Mg^{++}, Al^{+++}, and Fe^{+++}. The quinhydrone electrode was used in making the determinations.

SOILS USED

Six surface soils were chosen for the investigation. These soils had previously been used in base-exchange studies by Sewell and Perkins (12), and were selected as being representative of the locality in which they are found. The samples were collected in eastern Kansas, where the rainfall is between 30 and 40 inches a year, and the range in temperature varies approximately from $-20°C$. to $38°C$., although the extreme temperatures will be somewhat beyond these figures. The soils are mineral in nature and are somewhat acid, having been leached free from exchangeable sodium, and in most of them the iron has started to collect in concretions. The exchangeable base content of these soils, as obtained from the field, was determined by Perkins (12) and is shown in table 1. The methods for exchangeable bases are those of Gedroiz (6, 7).

[1] Contribution no. 154, department of chemistry.

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Since it was desirable to work with small samples, it was decided to use only the finer portions of the soil.   These portions were separated by sedimentation after the soil was saturated with sodium by double exchange, normal sodium chloride being used.   The fraction used was that fraction which, when freed from soluble chlorides, would remain in suspension in a 24-inch column of water for 24 hours, containing the particles of smaller diameter than 0.005 mm.   By this method approximately 20 per cent of the soil was separated for use.   This point of division was selected, as tests on these soils indicated that particles of this size and smaller contained almost all of the base-exchange complex.   The larger particles, having but a trace of exchange capacity, were regarded as inactive, and were discarded.   The use of only the finer particles facilitated the obtaining of small, uniform samples.

TABLE 1

Bases extracted by 0 05 N HCl, hydrogen by N BaCl$_2$, and pH of soils studied

SOIL NUMBER	SOIL TYPE AND SOURCE	pH, MOIST SOIL	EXTRACTED BASES IN M EQ PER 100 GM					
			H	Al	Fe	Ca	Mg	K
1	Summit, Ft Scott, Bourbon County	5 46	2 2	27	9 0	120	92	2
2	Cherokee, Columbus, Cherokee County	4 78	9 2	62	3 7	48	30	2
6	Boone, Halls Summit, Coffee County	5 66	?	21	4 3	94	89	4
8	Oswego, Moran, Allen County	5 46	7 2	53	1 3	63	31	2
11	Oswego, Manhattan, Riley County	?	?	18	1 0	187	24	1
12	Derby, Manhattan, Riley County	?	?	70	2 3	244	47	2

PREPARATION OF SAMPLES

The finer fractions of the soil as separated contained sodium as their exchangeable base.   These fractions were treated with the chlorides of various bases.   The soils were treated with normal chloride solutions of potassium, ammonium, calcium, magnesium, iron (ferric), and aluminum, and 0.05 N Hcl. These treatments were made by shaking approximately 20 gm. of soil with 500 cc. of the chloride solution in centrifuge bottles.   After thorough mixing was achieved the soil was packed by centrifuging and the supernatant liquid was poured off.   The electrolitic solution withdrawn was replaced and the process repeated until after equilibrium was reached.   In testing for equilibrium, determinations were made of the ammonium concentration in the ammonium chloride solution before and after being mixed with the soil.   Ammonium was selected, as its energy of adsorption is less than that of the other bases, except potassium, and its determination is simple.   The strength of the hydrochloric

freshly prepared soil was used the aluminum soils were more acid at the 1:100 dilution than at the 1:1 dilution. The indication of these variations is that even though the soils are air-dry they are not in equilibrium.

TABLE 6

pH values of MgCl₂-treated soils

SOIL-WATER RATIO	SOIL NUMBERS						
	1	2	6	8	11	12	Average
1:1	6 36	6 22	6 32	6 19	6 32	6 32	6 29
1:2 5	6 36	6 42	6 49	6 36	6 59	6 59	6 47
1:10	6 62	6 75	6 82	6 57	6 92	6 80	6 75
1·100	6 56	6 69	6 75	6 56	6 82	6 75	6 69

TABLE 7

pH values of CaCl₂-treated soils

RATIO	SOIL NUMBERS						
	1	2	6	8	11	12	Average
1:1	6 49	6 56	6 19	6 29	6 61	6 36	6 42
1:2 5	6 69	6 95	6 46	6 72	6 79	6 56	6 69
1.10	6 95	7 29	6 75	7 12	7 02	6 89	7 00
1.100	6 92	7 22	6 74	7 05	7 02	7 09	7 01

TABLE 8

pH values of KCl-treated soils

RATIO	SOIL NUMBERS						
	1	2	6	8	11	12	Average
1:1	6 72	6 72	6 82	6 75	6 68	6 78	6 74
1:2 5	6 80	6 85	7 05	6 78	7 07	7 25	6 97
1:10	7 30	7 40	7 58	7 43	7 72	7 78	7 54
1:100	7 72	7 68	7 58	7 55	7 78	7 92	7 71

TABLE 9

pH values of NaCl-treated soils

SOIL-WATER RATIO	SOIL NUMBERS						
	1	2	6	8	11	12	Average
1:1	6 68	6 72	6 85	6 75	6 92	6 98	6 82
1:2 5	6 98	6 92	7 02	6 88	7 15	7 32	7.04
1:10	7 68	7 58	7 85	7 65	7 99	8 02	7 80
1:100	7 78	7 75	8 02	7 72	8 22	7 95	7 92

From the data presented it may be judged that regardless of the base adsorbed by the soil the effect of dilution on soil reaction is similar. In the graph it may be noted that moderate dilution tends to increase alkalinity. It is also considered that a ratio of 1:2.5 is satisfactory for making pH determinations. A smaller amount of water with many soils gives a viscous paste that is hard to bring into intimate contact with the electrode, and a larger amount of water departs further from natural soil conditions.
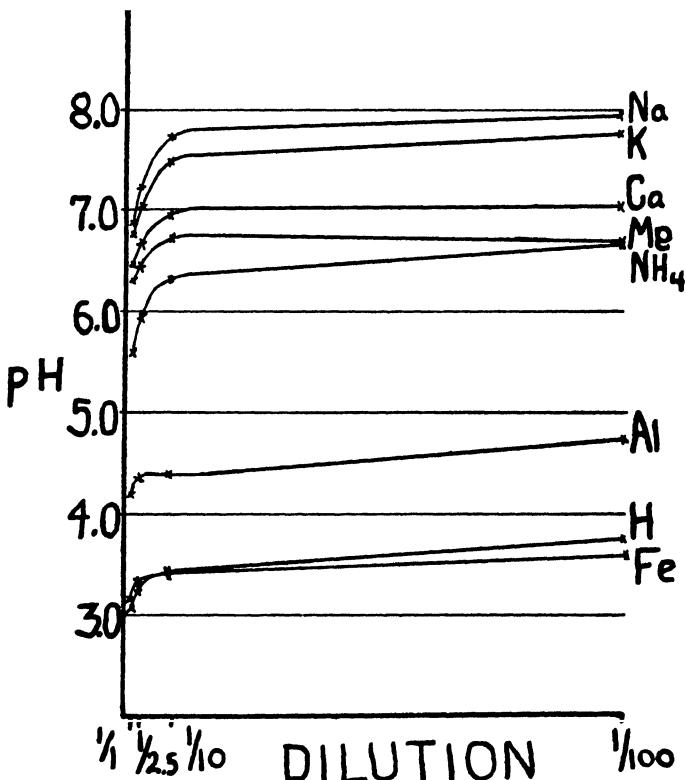
Fig. 1. pH Values at Several Soil-Water Ratios
Averages for six soils

It will be noted that maximum difference in pH values for dilution of 1:1 and 1:100 is 1.10 in the case of sodium and 0.40 in the case of magnesium. It will also be noted from the graph that regardless of base the curves between the dilutions of 1:1 and 1:2.5 are nearly vertical, and that between the dilutions of 1:10 and 1:100 are nearly horizontal.

The observations should be made that the soils treated with aluminum chloride have a low specific gravity and a high water holding capacity. In these

soils at the 1:1 dilution a thick paste was made that was hard to bring into contact with the electrode. The soils treated with other bases were much more fluid at this concentration.

As pH determinations were made on these soils from time to time it was noted that they became more acid as drying progressed. Work has started to bring portions of these soils into a state of dryness obtainable by storing them in a desiccator over calcium chloride.' Other portions have been stored in desiccators over water. Preliminary work has indicated that it will take about a year before these soils reach approximate equilibrium. When stability is obtained, with the recent publication of the International Society of Soil Science (11) in mind, new determinations will be made to find the effect of base adsorbed and degree of hydration on pH value and potential drift. Preliminary work indicates the possibility that the hydrolysis constant of the base associated with degree of dilution might have some effect on potential drift.

SUMMARY AND CONCLUSIONS[2]

Selected soils were treated with various bases and the pH values determined at several degrees of dilution.

It is found in working with eastern Kansas mineral soils that dilution tends to give a more alkaline reaction.

Regardless of the adsorbed base present in a soil, degree of dilution has about the same effect on the soil reaction.

A dilution of 1:2.5 is satisfactory for determining soil reaction.

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[2] *Note during printing:* Since the present article has been prepared for publication the abstract of the article by Aarnio (1) has been noted. Although the results presented here are not in complete accord with Aarnio's it is hoped that future work will explain these differences.

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A METHOD FOR THE STUDY OF AZOTOBACTER AND ITS APPLICATION TO FERTILITY PLOT SOILS[1]

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A great many investigations have been conducted on the physiology of the nitrogen fixing bacteria but few have been made on the effect of environmental conditions in the soil on these microorganisms. The object of the work reported in this paper has been to develop a method for comparing the Azotobacter flora in different soils, and to study the effects of various fertilizer treatments on the Azotobacter flora in fertility plot soils.

INTRODUCTION

Until very recently all studies on Azotobacter have been based on either the soil incubation method or the solution method.

The soil incubation method

A quantity of soil, usually 100 gm., is mixed with 1 or 2 per cent of mannite and placed in a tumbler or soup plate. Sufficient water is added to establish optimum moisture conditions. The soil is incubated for three or four weeks at a temperature of 25 to 28°C. The nitrogen content of the incubated soil and of the original soil is then determined. The difference between the nitrogen content of the incubated soil and that of the original soil represents the amount of nitrogen fixed.

The solution method

The culture solution contains a carbohydrate, nutrient salts, and an excess of calcium carbonate, but is deficient in nitrogen. Measured 50- or 100-cc. portions of this solution are put into 250-cc. Erlenmeyer flasks. The flasks of medium are sterilized and then inoculated with 5 or 10 gm. of the soil to be studied. Examinations for the typical brown Azotobacter film are made at

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intervals of a few days. The solutions are incubated for a total period of three or four weeks at 25 to 28°C. At the end of the incubation period total nitrogen determinations are made on the incubated cultures, on the control solution, and on the soil. The amount of nitrogen fixed is the difference between the nitrogen contained in incubated culture and that contained in the soil and the control solution.

The Winogradsky method

In 1926 Winogradsky (9) described a new method by which soils could be classified according to their Azotobacter flora. The method includes the five determinations listed below.

1. Silica gel plates, 9 cm. in diameter, are inoculated with 50 soil granules each. After 48 hours colonies develop about the granules which contain viable Azotobacter cells.

2. One-half gram of mannite is mixed with 50 gm. of moist soil. A microscopic examination is made after 48 hours' incubation.

3. Five per cent of starch is mixed with moist soil and the soil is molded into Petri dishes. The colonies which appear on the smooth surface of the soil are counted after 48 hours' incubation.

4. Silica gel plates, 20 cm. in diameter, are inoculated with 1 gm. of soil. The colonies appearing on the plates are counted after 48 hours' incubation.

5. The nitrogen fixed on the 20-cm. plates is determined after 5 days' incubation.

Limitations of these methods

The soil incubation method is unsatisfactory because the quantities of nitrogen fixed are usually too small to permit significant comparisons to be made. Any toxic substances produced during incubation accumulate and may retard Azotobacter activity before the end of the incubation period. Azotobacter is forced to compete with many other organisms for the carbohydrate provided. The method is not comparable to field conditions since the addition of 2 gm. of mannite to 100 gm. of soil is equal to a field application of 20 tons an acre. Such a large amount of available carbohydrate is never present under field conditions. Consequently, the rate of fixation under field conditions cannot be expected to compare with that obtained by this method.

There are a number of objections to the use of the solution method. With this method aerobic conditions are found only at the surface of the culture solution. Butyric acid is frequently formed during incubation and may retard or stop Azotobacter development. Azotobacter is forced to compete with other organisms for the mannite, and in addition, amoebae flourish in the solution and may devour many Azotobacter cells. It is very probable that a film is produced only when there are many Azotobacter cells present (3, 9).

Either the soil incubation method or the solution method thus provides a qualitative test for the presence or absence of Azotobacter, but neither provides for an estimation of the number present or a quantitative measure of their activity. The results obtained may depend as much on the variable factors in the methods as on the number or activity of the Azotobacter present.

No reports on the Winogradsky method were available when this project was begun in October, 1926. It appeared that it should be possible to make quantitative comparisons by this method. For this reason it was selected for further study.

<center>EXPERIMENTAL</center>

Laboratory studies were made on the five determinations included in the Winogradsky method. The first three of these determinations permit only superficial comparisons of the flora in different soils. They are essentially qualitative in nature and were found unsatisfactory for this investigation. The fourth and fifth include a determination of the number of Azotobacter colonies in the soil and a determination of the amount of nitrogen fixed under controlled conditions. Work was confined to these latter determinations since it was felt that only these would permit quantitative comparisons of the Azotobacter flora in different soils.

A detailed description of Winogradsky's procedure for these determinations follows.

Silica gel is used as a base for the medium in this determination and is prepared as follows:

Dilute hydrochloric acid, specific gravity 1 10, and potassium silicate solution, specific gravity 1 06, are mixed in equal volumes by pouring the silicate solution into the acid and shaking well This mixture is distributed immediately into 20-cm Petri dishes at the rate of 200 cc. per dish The layer of gel should be more rather than less than ½ cm. in thickness The dishes are left undisturbed on a level surface for at least 24 hours The gel is sufficiently solid when distinct vibrations are felt if the dish is tapped with the hand This indicates that the gel is sufficiently elastic and tough to undergo the subsequent treatment without being disrupted. The plates of gel are washed in running water for two or three days and finally with boiling distilled water by flooding the surface of the plates several times This wash water should give a distinct violet reaction with brom-cresol-purple and should give no turbidity with silver nitrate The dishes thus prepared may be kept in stock for a long time if they are protected from dessication

The following stock solution is prepared:

Di-potassium phosphate	0 5 gm
Magnesium sulfate	0 3 gm.
Sodium chloride	0.3 gm.
Ferric sulfate	Trace
Manganese sulfate	Trace
Distilled water	100 cc.

To prepare a nutrient plate, 10 cc. of this solution, 2 gm. of mannite, and 0.5 − 0.7 gm. of calcium carbonate are placed in a small flask, heated to boiling, and poured over the surface of the gel. The open plate is then dried at a temperature of 40 to 50°C. for several hours or until the surface liquid has evaporated. In order to prevent cracking of the gel the drying is discontinued as soon as the surface of the gel appears dry.

A representative sample of soil is collected from the plot to be studied. This sample should be a composite of a large number of borings taken at equal distances over the surface of the whole plot. This composite sample is carefully mixed and passed through a sterile 20-mesh sieve. About 12 gm. of the moist, sifted sample is used to determine moisture content by drying for two hours at 100°C. In the meantime the remainder of the sample is protected from drying. After the moisture content has been determined, the proper amount of moist soil to equal 1 gm. of dry soil is weighed out. This sample of moist soil is scattered uniformly over the surface of the gel.

The Azotobacter colonies may be recognized at the end of 48 hours' incubation at 28 to 30°C. In diameter and in thickness they form the only important growth on the plates. The number of colonies which have developed at this time are counted and are considered to represent the number of Azotobacter colonies per gram of soil. The nitrogen fixing activity of these bacteria may be determined on these same plates. For this purpose the incubation is continued to a total period of five days. At the end of this time the plates are dried at a gentle heat (40 to 50°C.) until the gel becomes a mass of dry, brown scales. The dried gel and the bacterial growth are then transferred to a Kjeldahl flask and the total nitrogen is determined. It is also necessary to determine the nitrogen content of a plate of uninoculated gel and of 1 gm. of the dry soil inoculum. The amount of nitrogen fixed is determined by difference.

This procedure may be criticized because the medium is not sterilized and because difficulty might be anticipated in identifying the Azotobacter colonies in the heterogeneous flora appearing on the plates. These criticisms are unwarranted. The medium is so sharply selective that in appearance the plates frequently suggest a pure culture. In each case the Azotobacter colonies stand out so sharply that they may be recognized by their macroscopic appearance alone. A microscopic examination impresses one with their uniformity and purity. It should be noted that the number of colonies which develop on the plates may not and probably does not correspond to the number of cells in a gram of soil since Azotobacter is found in the soil in the state of natural colonies. Consequently the number of viable colonies, which is probably much less than the number of cells, will determine the number of colonies that appear on the plates. This procedure is simple and rapid. Although it is not a pure culture method of study, it has produced remarkably constant results.

Development of the agar plate method

Soils from several plots of the Ohio Agricultural Experiment Station farm at Wooster were used in a preliminary study of the silica gel plate method. In general the results obtained were satisfactory. The soil remained on the surface and absorbed water from the gel. Aerobic conditions and yet an adequate supply of moisture were thus assured. The medium was found to be very selective and permitted the growth of typical and practically pure cultures of Azotobacter. No appreciable growth of other organisms was found

about the soil granules which did not produce Azotobacter colonies. Distinct differences were found between the different plot soils, the nitrogen fixed having varied from 0 to 17 mgm. per plate. The results were sufficiently uniform to permit a quantitative comparison of the Azotobacter flora of different soils.

Use of soil granules.—One objection to the Winogradsky method which early became evident is the type of inoculum used for the plates. The inoculum consists of soil which has been passed through a 20-mesh sieve. A great variation in the size and number of the soil granules is possible with such a soil sample. It was considered advisable to obtain a sample of granules more uniform in size. This has been done by passing the soil sample through two sieves, a 20-mesh sieve and a 40-mesh sieve. The soil granules which pass through the former but are retained on the latter are used as an inoculum. This restriction of the size of the soil granules has resulted in more uniform colony counts. A few counts of the soil granules per gram of soil from the different plots have been made. Although some variation in the number has been found, the differences have not been sufficiently great to account for the variation in the Azotobacter flora of the plots.

It is realized that an arbitrary standard has been set in the use of these granules but the uniform and consistent counts obtained have apparently justified its use on this particular soil type.

Use of agar.—The most serious objection to the method is due to the variation in the moisture content of the gel of different plates. Since the excess water is evaporated from the gel until the surface appears dry, the moisture content of different plates may vary considerably. On plates having a relatively low moisture content the colonies are somewhat viscous and maintain their identity. A high moisture content results in liquid colonies that have a marked tendency to coalesce. In the latter case they may spread until the growth covers the entire surface of the plate. The coalesence of the colonies makes a count difficult or impossible and causes considerable variation in the amount of nitrogen fixed. The length of time required to complete an analysis is another undesirable feature of the method. Approximately 10 days are required from the time the gel is prepared until the nitrogen analyses are completed.

In an effort to prevent the coalescence of colonies $1\frac{1}{2}$ per cent agar was substituted for the silica gel medium. In general this change has been very satisfactory. The preparation of the plates is more simple and much quicker since the medium may be prepared and the plates inoculated in a few hours' time. The colonies do not develop as rapidly on the agar medium as on the silica gel. Consequently, an incubation period of three or four days at 28°C. is necessary to develop the maximum number of colonies. The more uniform water content of the agar as compared with the silica gel results in a marked reduction of the tendency of the colonies to coalesce. Thus, more uniform results are obtained, both in colony counts and in nitrogen fixation. The agar medium is slightly less selective than the silica gel, but the Azotobacter colonies

are easily distinguished from the few other types which occasionally appear on the plates.

Selection of nutrients.—Attempts were made to replace mannite with a less expensive source of energy. The Azotobacter colony count remained practically the same when mannite, dextrose, or dextrin was used. A medium prepared with either dextrose or dextrin, however, was found to be much less selective than the mannite medium. The use of mannite has been continued because it is difficult to identify the Azotobacter colonies when using dextrose or dextrin.

The stock solution used by Winogradsky is quickly contaminated by molds so it has been replaced by a dry stock salt mixture. The following proportions of salts are ground to pass a 40-mesh sieve, thoroughly mixed, and stored in a glass-stoppered jar until used.

	Parts
Di-potassium phosphate	100
Magnesium sulfate	60
Sodium chloride	60
Ferric sulfate	1
Manganese sulfate	1
Calcium carbonate	178

Two grams of this mixture is used for each liter of medium.

Use of aluminum dishes —The 20-cm. glass Petri dishes used in this procedure are rather unsatisfactory. They are clumsy and heavy to handle in quantities Dishes of the grade usually available have irregular bottoms. For this reason it is necessary to use a large quantity of medium to obtain a sufficiently uniform layer of gel. The glass plates cannot be stacked in the quantities desirable for sterilization or storage. Sterilization and handling of plates cause considerable breakage, and replacements are expensive.

To avoid these undesirable features of the glass Petri dishes, dishes made of aluminum were substituted [3] The aluminum dishes have proved to be very satisfactory for this type of work. These dishes weigh less than half as much as the glass dishes. They are uniform in shape, have flat tops and bottoms, and may be stacked for sterilization or storage. Satisfactory photographs of single colonies or entire plates can be made from cultures on the aluminum dishes. There is no danger of breakage in sterilization or in other handling, and the original cost is decidedly less than the cost of glass dishes.

Proper incubation period —The proper incubation period and the number of plates necessary to obtain significant results were determined by the following experiment. Fifty plates were inoculated with a plot soil known to have a relatively low Azotobacter count. These plates were counted after two, three, four, five, and six days' incubation. The results are shown in figure 1. From this figure it is evident that an incubation period of at least three days is

[3] The aluminum petri dishes were made available through the courtesy of The Buckeye Aluminum Company, Wooster, Ohio. They may be obtained by ordering Style No. 8015.

necessary to obtain a significant colony count. The apparent slight decrease in the count after five days' incubation is due to the coalesence of the colonies as they grow larger.

Number of plates required.—The probable error of the plate count was first determined. The count for the foregoing series of 50 plates after four days incubation was found to be 16.6 ± 0.34 colonies per gram, an error of 2.07 per cent (7). The percentage of error for various numbers of plates was then calculated according to the formula

$$Em = \frac{0.6745 \times C.V.}{\sqrt{n}}$$

where Em is the percentage of probable error, $C.V.$ is the coefficient of variability, and n is the number of determinations. The percentage of error is
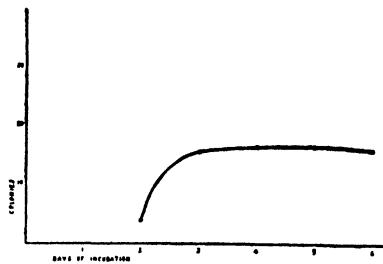
<div align="center">Fig. 1   Fig. 2</div>

FIG. 1. RELATION OF INCUBATION PERIOD TO NUMBER OF COLONIES
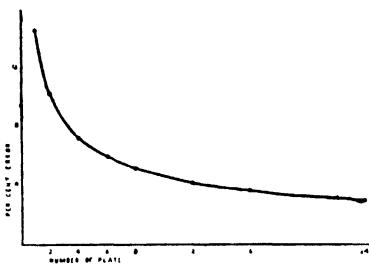
FIG. 2. RELATION OF NUMBER OF PLATES TO PER CENT ERROR

plotted against the number of plates in figure 2. This graph indicates that the probable error may be maintained within 5 per cent by the use of eight plates for each soil sample. Eight plates have been used in all subsequent work and the differences between the counts for different plots have been found to be significant in practically all cases.

Relation of colony count to nitrogen fixation.—Winogradsky used the 20-cm. plates to determine: first, the number of Azotobacter colonies per gram of soil; and second, the ability of the soil to fix nitrogen. He evidently believed that the determination of the nitrogen fixed would yield information in addition to that given by the colony count. The nitrogen fixed has been found, however, to be a function of the number of colonies per plate. This relation obviates the determination of nitrogen fixation on the plates. For this reason nitrogen determinations have been discontinued in all subsequent work.

To determine this relation, the data obtained from a series of 167 plates were tabulated according to the number of colonies per plate. The nitrogen fixed per colony was then calculated from the nitrogen fixed per plate. The nitrogen fixed per colony appeared to decrease as the number of colonies per

plate increased. To summarize these data they were divided into groups according to the number of colonies per plate as follows: 1–5, 6–10, 11–15, 16–20, etc. The average colony count and the average nitrogen fixation per colony were determined for each of these groups. The values obtained are given in table 1.

The nitrogen fixed per colony plotted against the number of colonies per plate is shown in figure 3. It is evident that the nitrogen fixed per colony decreases as the number of colonies per plate increases, and although the relation is not linear it tends to follow a smooth curve. From the general positions of the points it might be expected that a curve drawn through them would assume the form of a logarithmic curve. To determine the "best" curve that

TABLE 1

Comparison of colony count with nitrogen fixation

NUMBER OF PLATES	COLONIES PER PLATE	AVERAGE NUMBER OF COLONIES	NITROGEN FIXED PER COLONY		ERROR
			Determined	Calculated	
			mgm	*mgm*	*per cent*
36	2–5	3 5	0 234	0 242	−3 3
12	6–10	7 5	0 227	0 207	+10 3
7	11–15	13 0	0 203	0 182	+11 5
14	16–20	18 4	0 153	0 166	−7 8
12	21–25	22 8	0 151	0 156	−3 2
10	26–30	28 1	0 138	0 146	−5 5
11	31–35	33 6	0 131	0 138	−5 1
11	36–40	38 0	0 134	0 132	+1 5
13	41–45	43 2	0 153	0 127	+20 5
7	46–50	48 0	0 128	0 122	+4 9
10	51–55	52 5	0 122	0 118	+3 4
6	56–61	57 3	0 116	0 113	+2 7
6	97–132	115 2	0 088	0 081	+8 6
6	147–178	156 0	0 051	0 067	−23 9
6	201–232	218 0	0 047	0 052	−10 4

could be drawn through these experimental points the procedure outlined in the following was carried out.

If the relation were truly logarithmic, one would expect to obtain a straight line if the nitrogen fixed per colony were plotted against the logarithm of the number of colonies per plate. This is shown in figure 4. The equation of the "best" line through these experimental points, determined by the method of least squares, is

$$y = -0.1061 \log x + 0.300$$

where y is the milligrams of nitrogen fixed per colony and x is the number of colonies per plate. With this equation as a basis, the positions of a number of points on the line were calculated. These calculated values are given in

table 1. The "best" line through the points in figure 3 and in figure 4 was drawn from these calculated values. The experimental points are seen to conform closely to this calculated logarithmic curve. Rather than a direct linear relation existing between the nitrogen fixed per colony and the number of colonies per plate, apparently the relation is one in which the nitrogen fixed is an inverse function of the logarithm of the number of colonies per plate. It should be noted that this relation may be expected only if the experimental conditions are maintained within definite limits. These limits have not been determined.

Procedure adopted.—The revised procedure now used is carried out as follows.

The medium is made up of 15 gm. of agar, 20 gm. of mannite, and 2 gm. of the stock salt mixture for each liter of medium. This is heated in the autoclave until the agar is completely dissolved. Into each 20-cm. aluminum Petri dish 100 cc. of the medium is poured and allowed to harden. One gram of soil granules that pass a 20-mesh sieve but are retained by a 40-mesh sieve
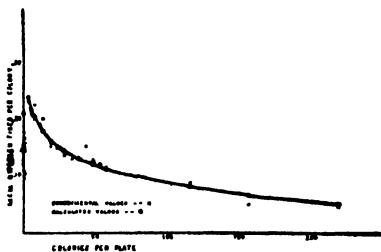
FIG. 3   FIG. 4

FIG. 3. RELATION OF COLONY COUNT TO NITROGEN FIXATION
FIG. 4. RELATION OF LOGARITHM OF COLONY COUNT TO NITROGEN FIXATION
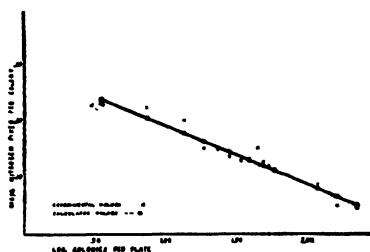
is scattered as uniformly as possible over the surface of the agar. Eight plates are inoculated with each soil to be analyzed. After four days' incubation at 28°C. the colonies which have developed on the plates are counted. The average of the counts on the eight plates is taken as the Azotobacter colony count per gram of the soil tested.

Application of the method

The agar plate procedure has been applied to soil samples from the plots of the 5-year rotation fertility experiment at Wooster. The soil on sections C and D of this rotation is Wooster silt loam, a soil formed by the weathering of glacial drift derived largely from sandstone and shale. The surface soil is a brown silt loam which contains some sandstone fragments. This is underlain by a yellowish-brown silt loam only slightly heavier than the surface soil. This soil is acid except where limed, the reaction usually being about pH 5.0. The rotation consists of corn, oats, wheat, clover, and timothy. The plots were laid out in 1893 and have received consistent fertilizer treatment since

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that time (6). One end of each plot has received lime at 5-year intervals whereas the other end has never received lime.

TABLE 2

Colony counts for section C

PLOT	TREATMENT	LIMED						UNLIMED	
		pH	Colonies per gram				pH	Colonies	
			Spring, 1928	Fall, 1928	Spring, 1929	Average		Spring, 1928	
1	Check	7 4	245	145	94	161	5 2	0
2	P	7 4	137	54	38	76	5 0	0
3	K	7 5	108	39	34	60	4 9	0
4	Check	7 6	17	48	43	36	4 9	0
5	N	7 6	156	149	101	135	5 2	0
6	P–N	7 5	90	77	123	97	5 1	0
7	Check	7 4	116	87	102	102	4 9	0
8	P–K	7 4	66	80	50	65	4 8	0
9	K–N	7 4	173	207	136	172	4 9	0
10	Check	7 4	161	101	208	157	5 0	0
11	P–K–N	7 4	79	101	86	89	5 0	0
12	P–K–N	7 3	116	98	30	81	5 1	0
13	Check	7 4	168	168	184	173	4 9	0
14	P–K–N	7 4	162	137	86	128	4 8	0
15	P–K–N	7 3	88	122	71	94	4 9	0
16	Check	7 2	195	76	46	106	5 0	0
17	P–K–N	7 6	23	45	23	30	4 9	0
18	Manure	7 6	66	44	15	42	4 9	0
19	Check	7 5	249	128	158	178	4 9	0
20	Manure	7 4	58	83	35	59	5 0	0
21	P–K–N	7 3	145	99	..	122	4 9	0
22	Check	7 4	194	92	88	125	4 9	0
23	P–K–N	7 2	70	75	. .	73	4 9	0
24	P–K–N	7.1	6	18	. .	12	4 8	0
25	Check	7.5	456	58	. .	256	4 9	0
26	P–K–N	7 5	53	66	.	60	4 9	0
27	P–K–N	7 5	47	37	..	42	4 9	0
28	Check	7 6	169	205	...	187	5 1	0
29	P–K–N	7.6	346	52		199	5 2	0
30	P–K–N*	7 7	750	199	...	475	6 0	1

' No treatment since 1921.

The Azotobacter colony counts of the plots of section C and D, determined at different times, and the reactions of the plots in the spring of 1928 are given in tables 2 and 3.

TABLE 3

Colony counts for section D

PLOT	TREATMENT	LIMED							UNLIMED	
		pH	Colonies per gram						pH	Colonies
			Fall, 1927	Spring, 1928	Fall, 1928	Spring, 1929	Average			Spring, 1928
1	Check	7 8	219	243	181	140	196		5 1	0
2	P	7.5	156	115	74	198	136		5 1	0
3	K	7 4	42	27	42	77	47		5 0	0
4	Check	7.4	115	98	67	314	148˙		5 0	0
5	N	7 6	54	69	46	83	63		5 1	0
6	P–N	7 4	31	32	11	21	24		5 0	0
7	Check	7 4	20	26	37	23	27		4 9	0
8	P–K	7 2	4	4	7	10	6		5 0	0
9	K–N	7 5	31	19	10	21	20		5 0	0
10	Check	7 3	49	90	57	177	93		4 9	0
11	P–K–N	7 3	17	29	16	37	25		5 0	0
12	P–K–N	7 4	20	13	24	34	23		5 1	0
13	Check	7 3	32	15	36	12	24		4 9	0
14	P–K–N	7 2	3	2	4	2	3		5 1	0
15	P–K–N	7 2	1	5	2	1	2		5 2	0
16	Check	7 4	45	51	38	17	38		5 1	0
17	P–K–N	7 5	1	1	25	1	7		5 1	0
18	Manure	7 4	4	2	3	15	6		5 1	0
19	Check	7 6	34	50	56	87	57		5 1	0
20	Manure	7 5	47	60	31	37	44		5 2	0
21	P–K–N	7 4	13	5	11		10		5 0	0
22	Check	7 5	41	38	42	66	47		5 0	0
23	P–K–N	7 4	5	8	13	.	9		5 0	0
24	P–K–N	7 3	2	1	3		2		4 8	0
25	Check	7 6	20	20	37	. .	29		5 0	0
26	P–K–N	7 4	6	12	12	.	10		5 2	0
27	P–K–N	7 5	5	3	15	. .	8		5 0	0
28	Check	7 4	31	36	24	.	30		4 9	0
29	P–K–N	7 5	8	3	9	. . .	7		5 2	0
30	P–K–N*	7 5	54	72	52	.	59		5 0	0

' No treatment since 1921.

Discussion of data for section C.—The average of three series of Azotobacter colony counts on the limed plots of section C is presented in column 7, table 2.

In this table since the check plots are given first in each series a comparison can readily be made between the treated plot counts and their check values. In most cases the counts on the check plots are much greater than the counts on the adjacent treated plots. The average of the counts on the check plots is 177 colonies per gram whereas the average of the counts on the treated plots is only 86 colonies per gram. A statistical analysis shows that the odds are 13,000:1 that the difference between these two averages is not due to chance alone and may be considered significant (5).

Three plots, 5, 6, and 9, in this section have a higher colony count than their respective checks. On referring to the fertilizer treatments on these plots it is observed that each of them receives nitrate of soda, either alone or in combination with superphosphate or muriate of potash. In order to make a
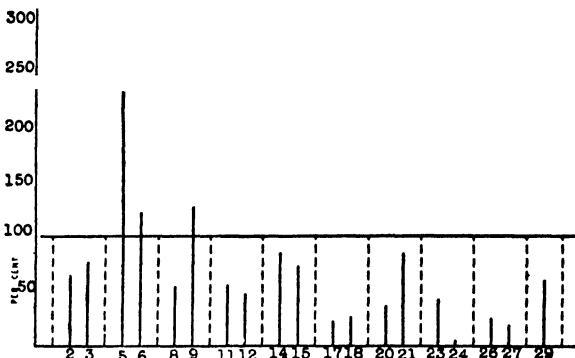
FIG. 5. DATA FOR SECTION C ON CORRECTED CHECK BASIS

further study of these plots the data in column 7 of table 2 have been calculated on a corrected check basis as shown in figure 5.

The check value for each plot has been calculated upon the assumption that the variations in the soils are progressive; that is, if the counts on plots 1 and 4 were 100 and 70 colonies, respectively, then the counts on plots 2 and 3, if left unfertilized, should be 90 and 80, respectively (6). The colony count of each treated plot has been expressed in terms of per cent of its check value in figure 5. The check value is indicated by the line at 100 per cent. The general relation between treated plots and check plots is evident. The average Azotobacter colony count on the treated plots, even including plots 5, 6, and 9 which have exceptionally high counts, amounts to only 67 per cent of the corrected check value. This indicates that, in general, the Azotobacter population has decreased subsequent to fertilizer treatment.

The plots which receive either one or two fertilizer element treatments are of special interest. These are plots 2, 3, 5, 6, 8, and 9. Plot 5 receives applica-

tions of nitrate of soda and the count amounts to 232 per cent of its check value. Thus it appears that nitrate has had a distinct stimulating action on Azotobacter growth. This is in accord with the findings of Hills (4), who reported that Azotobacter was stimulated by small amounts of potassium, sodium, or calcium nitrate.

Plot 2 receives superphosphate and the count amounts to 64 per cent of the check value. Plot 3 receives muriate of potash and the count is 77 per cent of the check value. According to the counts obtained on these two plots it appears that additions of either superphosphate or muriate of potash have been followed by a decrease in the number of Azotobacter colonies. A statistical comparison of the populations on plots 2 and 5 was made and it was found that the odds are 49:1 that plot 5 has the greater Azotobacter population. A similar comparison of plot 3 with plot 5 indicated the odds to be 36:1 that plot 5 has the greater population. From these comparisons it appears that nitrate
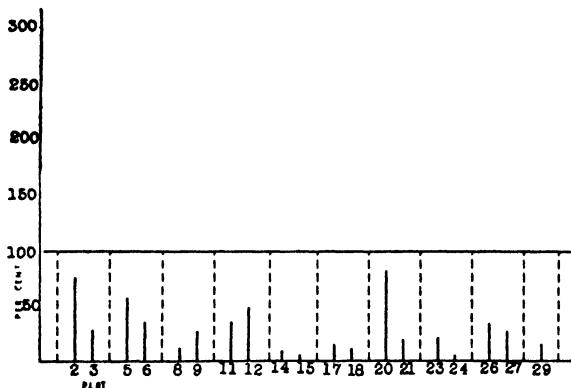
FIG. 6. DATA FOR SECTION D ON CORRECTED CHECK BASIS

of soda is more favorable to the growth of Azotobacter than is either superphosphate or muriate of potash.

In the case of plots 6, 8, and 9, each receives treatments which consist of two fertilizer elements. Plot 6 receives phosphate and nitrate, plot 8 receives phosphate and potash, and plot 9 receives potash and nitrate. Plots 6 and 9 receive nitrate with either phosphate or potash, and each has a count higher than its respective check value. Plot 8 receives phosphate and potash but no nitrate, and has a count of only 54 per cent of its check value. A comparison of plot 6 with plot 8, shows the odds to be 49:1 that plot 6 has a higher Azotobacter count than plot 8. A comparison of plot 9 with plot 8 shows that the odds are 13:1 that plot 9 has a higher count than plot 8.

From a study of the data for these six plots it would appear that additions of nitrate of soda have been followed by an increase in the number of Azotobacter colonies, whereas additions of superphosphate or muriate of potash have been followed by a decrease in the number of Azotobacter colonies.

The treated plots above plot 10 receive complete fertilizer additions and in all cases the Azotobacter count is less than the check value. Another experiment may be mentioned in connection with the foregoing statement. A study was made of the 4-year rotation liming experiment plots at Wooster (6). These plots receive a basic treatment of manure and superphosphate while the form and amount of liming material is varied from plot to plot. The counts obtained on all plots were so low that no significant comparisons could be made. These plots receive complete fertility treatment and therefore the low counts confirm the results obtained on the complete fertilizer and manure plots of sections C and D. It is particularly interesting to note that the counts are low on these plots in spite of the fact that some of them receive as much as 8 tons of lime per acre per rotation.

Discussion of data for section D.—The four series of counts made on section D are summarized in table 3, column 8. The average colony count for the check plots of this section is 68 colonies per gram whereas the average for the treated plots is 24 colonies per gram. No treated plot in this section has a higher count than its check value. In this section it has not been possible to make comparisons between the plots which receive one and two-element fertilizer additions because the counts are abnormally high on the first few plots. These high counts may be due to the effects of a limestone road which runs parallel with and adjacent to plot 1.

Particular attention is called to plot 8 of this section. This plot receives superphosphate and potassium chloride but no nitrogenous fertilizer. The crop producing power of the plot has been maintained at approximately the same level through 35 years under this treatment. Since no nitrogen has been added to the plot it has been cited as an example of the ability of soil to fix nitrogen (1). Approximately one-half of this fixation has been ascribed to symbiotic organisms and the remainder to Azotobacter and related organisms. This plot is well drained, limed, supplied with phosphate, potash, and a fair amount of crop residues, conditions which apparently meet the requirements for abundant Azotobacter growth. In spite of these apparently favorable conditions the Azotobacter colonies are very few, an average of only 6 colonies per gram having been found (see plate 1). This number is so small that Azotobacter cannot be considered as an important factor in the maintenance of the fertility level of this plot.

The summary of the data from section D, calculated on a corrected check basis, is shown in figure 6. Even more distinctly than section C, does this section show a low colony count on the treated plots as compared with the check plots. The average of the colony counts on the treated plots amounts to 39 per cent of the corrected check value.

Discussion of data for check plots.—The variations in the counts on these check plots are wide. In section C the counts vary from 36 colonies per gram on plot 4 to 475 colonies per gram on plot 30. In section D the variation is from 24 colonies per gram on plot 13 to 196 colonies per gram on plot 1. The

counts on the last plots of section C and the first plots of section D are abnormally high as compared with the other plots of the sections. These high counts are apparently due to the effects of a limestone road which separates the two sections, although definite proof of the relation is lacking at the present time. No explanation is offered for variations in other parts of these sections. Obviously these uncontrolled and uncorrelated variations make an accurate interpretation of the results difficult.

An attempt was made to correlate the Azotobacter populations with the crop yields on the check plots by comparing the colony counts with the yields of each of the five crops of the rotation. No definite correlation, either positive or negative, could be demonstrated between the Azotobacter colony counts and the yields of any of the crops grown.

Discussion of data for manure plots.—It is generally believed that additions of manure to soil stimulate Azotobacter growth. This belief has not been substantiated in the data obtained by the agar plate method.

In section C, plot 18 receives 16 tons of manure per rotation and the Azotobacter colony count is 27 per cent of the corrected check value (see plate 1). Plot 20 receives 8 tons of manure and the colony count is 37 per cent of the corrected check value. In section D, plot 18 has a colony count of 12 per cent of its check value, and plot 20 has a count of 82 per cent of the check value. The 4-year rotation liming experiment receives a basic treatment of 8 tons of manure. The colony counts obtained have been so low that no comparisons could be made between different plots.

From the data obtained thus far it appears that manure has resulted in a depression rather than a stimulation of Azotobacter.

Discussion of data for unlimed plots.—Extensive studies on the presence of Azotobacter in soils of different reactions were made by Gainey (3) who found a critical reaction of pH 6.0 for the growth of this organism. In the spring of 1928 the unlimed ends of the plots of sections C and D were sampled and the samples analyzed for Azotobacter. Azotobacter was found to be absent in all but 1 of the 60 unlimed soils tested (tables 2 and 3). This soil was from plot 30 of section C and had a count of one colony per gram. This plot lies adjacent to the limestone road previously mentioned and has received enough lime from this source to raise the reaction to pH 6.0. The foregoing data have been obtained by a quantitative study on Azotobacter and tend to confirm the qualitative results obtained by Gainey.

It is believed that this paper presents the first quantitative data published concerning the effects of lime and fertilizer treatments on the growth and activity of Azotobacter colonies in soil.

SUMMARY

The agar plate method, an adaptation of the Winogradsky silica gel plate method for determining the number of Azotobacter colonies in soil has been developed, and factors influencing its use have been studied. A study of the

Azotobacter flora on the 5-year rotation plots at Wooster has been carried on during 1927, 1928, and 1929. The following statements appear to be justified by the data obtained:

In general the Azotobacter population has been much greater in the limed check plots than in the limed plots which receive fertilizer treatments.

Additions of nitrate of soda have been followed by greater growth of Azotobacter than additions of either superphosphate or muriate of potash.

Additions of manure have resulted in a depression of the Azotobacter flora.

It has not been possible to correlate Azotobacter populations and crop yields in the check plots.

Azotobacter colonies have not been found in soils which have a reaction more acid than pH 6.0.

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PLATE 1

RANGE OF COLONY COUNTS

SECTION D

PLOT 8
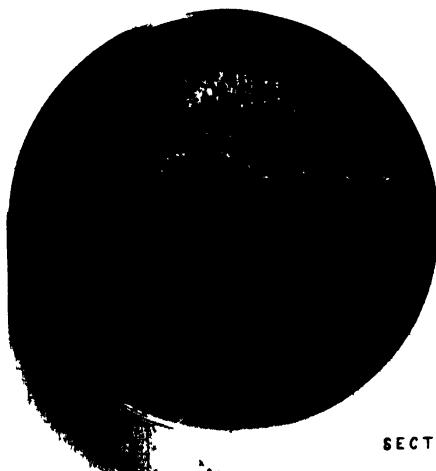
PLOT 18

SECTION C

PLOT 18

THE CARBON-ORGANIC MATTER FACTOR IN FOREST SOIL HUMUS

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It is generally recognized that the most reliable measure of the organic matter content of the soil is the amount of organic carbon present. Comparisons are then made either as carbon or else on the basis of organic matter obtained by the use of the factor 1.724.

As is well known, this factor was obtained by Schulze, Wolff, Van Bemmelen, and others (3, 4, 5, 8) in the latter part of the nineteenth century and has been used rather freely since then. It is based on the assumption that the carbon content of soil organic matter is 58 per cent. Some doubts have been raised from time to time as to the advisability of applying the factor too generally. Read and Ridgell (5) from a study of 37 soils came to the conclusion that if a factor is to be used it should be based on 51 or 52 per cent carbon rather than 58 per cent. They preferred using nitrogen instead of carbon as a basis for calculating the organic matter content. Leighty and Shorey (4), and more recently Waksman (8), maintain that the equation, $N \times 20 = O. M.$, is untrustworthy for general use. Waksman states that the method, $C \times 1.724 = O. M.$, proposed by those earlier soil chemists is ". . . . still the most reliable that we have at the present time for measuring the total organic matter of the soil."

The foregoing discussion is meant to refer primarily to the mineral layers of the average soil. The question arises, is the factor 1.724 equally applicable to peat soils and to the organic horizons of forest soils?

Since, in the case of mineral soils, loss on ignition is not a reliable measure of the organic matter content, and since an exact method for this determination has not yet been found, it has been necessary, as Waksman states, to use a factor in connection with the organic carbon content. On the other hand, the loss-on-ignition data for organic materials such as peats and forest litter are a very close if not an exact measure of the total organic matter, and one need not depend upon the carbon analysis with a correcting factor. Nevertheless, such a factor is a convenience especially in making comparisons with other soil horizons.

As was mentioned previously, the factor 1.724 assumes a carbon content of 58 per cent. There is considerable evidence, however, to show that the carbon content of highly organic material such as peats and forest litter is less than 58 per cent and therefore the factor 1.724 is too low.

In 1916, Gortner (3) reported a factor of 1.842 for peats, and 1.971 for vege-

table materials such as alfalfa, oats, oak leaves, fern leaves, and grass. As he obtained these results from only a few samples he entertained the hope that some one would make further studies on the problem. Robinson, McLean, and Williams (6) found an average carbon percentage of 52.95 for four peats, giving a factor of 1.888. Alway and Harmer (1) working with forest floor material from forests in three different localities in Minnesota obtained the factors 1.86, 1.89, and 1.85. Two of the localities were in the deciduous forest belt, whereas the third was farther north in the coniferous region. These investigators included all of the dead material overlying the mineral soil, separation into litter and "F" and "H" layers not being made. They state:

The weight per acre of organic matter, computed from the organic carbon, is about one-eleventh less than the weight of volatile matter. This difference is probably due to the presence of so-called *water of constitution* which is driven off while the organic matter is undergoing oxidation, and may be due in part to the factor used—1.724—being too low.

EXPERIMENTAL

Forest soil investigations at the Connecticut Agricultural Experiment Station include the determination of the organic carbon and the ash of the organic horizons. Carbon analyses were made by either the wet combustion method (2) using the Meyer bulb tube instead of the usual absorption tower, or the dry combustion method (2) using the Parr apparatus. Either method gave satisfactory results, but the former has been abandoned in favor of the latter as more suitable to this laboratory. Ashing was carried out according to the official methods.

It is our practice in detailed forest soil studies to recognize as many as three types of leaf material constituting the A_0 horizon: the freshly fallen leaves or *litter;* the partially decomposed and still decomposing "F" or *fermentation* layer often called "duff;" and the well-decomposed, structureless "H" or *humification*[1] layer.

When the data from the organic carbon analyses were plotted against that of the ash content excellent correlation was observed, as would be expected. It was seen at once, however, that the percentage of carbon in the organic matter falls somewhat short of 58 and the resulting factor $\left(\text{factor} = \dfrac{100}{\text{per cent C}} \right)$ would be greater than 1.724.

Furthermore, some scattering of the points was evident and when investigated it was found that a more or less distinct separation of the samples could be made, based on the three types of material mentioned in the foregoing. Dividing the loss-on-ignition (100—ash content) of each sample by its respective carbon content, we have an average factor of 1.892 ± 0.0057 for 14 samples of litter; 1.854 ± 0.0038 for the duff (55 samples); and 1.799 ± 0.0074 for the

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[1] The terms *fermentation* and *humification* were proposed at the 1930 meeting of the Association of Soil Survey Workers, to be applied to the horizons in forest soils which Hesselman has called the "F" layer, from the Swedish "Formultningsskiktet" and the "H" layer from the Swedish "Humusamneskiktet," respectively.

TABLE 1

Loss on ignition, carbon, and resulting factors of the several organic horizons of forest soils

$$\left(Factor = \frac{\text{Loss on ignition}}{\text{Per cent C}} \times 100 \right)$$

SOIL NUMBER	LOSS ON IGNITION	C	FACTOR
	Per cent	Per cent	
Litter material			
F 192	96 48	52 78	1.827
F 313	95 88	50.19	1.910
F 426	95 84	51 15	1 874
F 379	95 81	52 41	1.828
F 419	95 52	49 35	1 935
F 317	95 22	50 76	1 876
F 305	94 62	50 10	1 889
F 377	94 49	49 43	1 911
F 315	94 22	49 59	1.900
F 180	93 71	49 01	1 912
F 400	92 00	48 20	1 909
F 178	91 47	47 83	1 912
F 309	89 87	47 07	1.909
F 176	79 08	41 57	1.902
Average			1 892 ±0.0057
"F" or fermentation layers			
G 14	98 15	53 50	1 834
F 346	96 92	51 50	1.882
G 20	96 82	52 70	1.837
F 333	95 91	50 45	1 901
F 363	95 69	51 80	1 847
G 38	95 62	52 50	1.821
F 306	95 48	52 00	1.836
F 380	94 82	52 57	1 804
F 185	94 57	50 47	1 874
G 21	94 35	51 00	1 850
F 367	94 31	51 21	1 842
G 36	94 25	49 75	1.894
G 16	93 90	50 60	1.856
G 18	93 64	50 20	1.865
F 246	93 38	49 10	1 902
F 311	93 36	49 33	1 892
F 271	93 35	48 30	1.933
F 299	93 34	48 80	1.913
G 35	93 31	49 40	1.889
F 192.1	93 23	51 23	1.820
G 23	92 27	49 50	1.864
G 39	92 01	50 00	1.840
F 373	91 86	50 58	1.816

TABLE 1—*Continued*

SOIL NUMBER	LOSS ON IGNITION		
	Per cent	Per cent	

"F" or fermentation layers—Concluded

SOIL NUMBER	Per cent	Per cent	
G 30	91 54	49 40	1 853
G 17	91 50	48 80	1 875
G 32	91 49	49 45	1 850
G 33	90 95	49 50	1 837
F 200	90 85	48 73	1 864
G 37	90 83	48 30	1 880
F 32	90 55	49 20	1 840
F 51	90 00	48 80	1 844
F 427	89 79	48 20	1 863
S 358	88 50	47 50	1 863
F 420	88 28	46 20	1 911
F 119	88 28	46 70	1 890
F 239	87 60	46 55	1 882
F 44	87 52	48 70	1 797
F 9	87 09	49 10	1 774
F 207	87 00	45 80	1 899
F 407	86 80	46 55	1 865
F 285	86 72	45 50	1 906
F 76	86 17	45 45	1 896
F 112	84 72	44 90	1 887
S 362	84 50	45 50	1 857
F 103	84 00	44 75	1 877
F 91	83 93	47 01	1 785
F 265	81 00	42 80	1 892
F 97	78 65	41 55	1 893
F 106	77 35	42 15	1 835
F 25	76 10	42 30	1 799
F 17	70 30	41 05	1 712
F 109	68 75	37 90	1 814
F 69	67 50	37 50	1 800
F 413	66 56	36 22	1 838
F 38	55 60	31 08	1 789
Average			1 854 ±0 0038

"H" or humification layers

SOIL NUMBER	Per cent	Per cent	
F 193	96 21	53 97	1 783
F 186	92 85	49 00	1 894
F 5	91 55	52 75	1 736
F 6	91 36	52 80	1 730
F 18	86 80	49 50	1 754
F 381	85 53	49 35	1 734
F 318	83 98	47 22	1 778
F 10	83 90	48 20	1 741
F 45	82 63	46 37	1 782

TABLE 1—*Concluded*

SOIL NUMBER	LOSS ON IGNITION		
	Per cent	Per cent	
"*H*" or humification layers—Concluded			
F 307	81 40	46 68	1.744
F 33	80 90	43 40	1.864
F 311½	79 12	43 04	1 838
F 187	79 06	45 02	1 756
F 26	80 49	44 83	1 795
F 368	71 30	39 75	1.794
F 52	71 20	38 58	1.845
F 364	70 83	38 13	1.857
F 194	67 93	38 04	1 786
S 359	65 90	35 80	1.841
F 370	63 60	36 62	1.737
F 113	60 90	32 50	1 874
F 120	49 00	27 41	1 788
F 428	35 60	19 56	1 820
F 70	34 40	18 10	1 900
Average		.	1.799 ±0 0074

humus material (24 samples). The loss on ignition, carbon content, and the resulting factors are found in table 1.

Calculated as per cent carbon $\left(C = \dfrac{1}{factor} \right)$ the results are, for the litter 52.85; for the duff, 53.94; and for the humus material, 55.58 per cent.

The proportional increase in the carbon content with progressive decomposition is due to the disappearance of the pentosans and celluloses, which have a relatively low carbon content of 42-44 per cent, and the accumulation of lignins and synthesized cell material with a high carbon content, 62–64 per cent (7, p. 675, 8).

When the data for organic matter obtained by the old factor (C × 1.724) and those obtained by the proposed factors (C × 1.892, 1.854, and 1.799 for the litter, duff, and humus respectively) are compared with the figures for the loss on ignition, and the whole is subjected to statistical analysis, we have the following results:

	C × 1 724	C × New factor	Loss on ignition
Per cent organic matter (average of 93 samples)....	79 6	85 4	85 3

Coefficient of correlation[2] (Pearson's method)

Between C × 1 724 and loss on ignition	Between C × 1 892, 1 854, or 1 799 and loss on ignition
0.975 ±0.0035	0.983 ±0 0024

[2] Jones, D. F. 1925 Genetics in Plant and Animal Improvement, p. 198–201. John Wiley and Sons, Inc., New York.

The degree of correlation is seen to be considerably higher in the second case than in the first; also the probable error is less in the second case. This establishes beyond a doubt the greater reliability of the new factors.

These results substantiate those of Gortner (3), Robinson, McLean, and Williams (6) and Alway and Harmer (1), all of which indicate a lower carbon content than 58 per cent and a factor above 1.724 for materials high in organic matter.

In an endeavor to ascertain whether or not the factors might differ for different forest types, a separation was made into the following classes: young hardwoods (40 years or less), mature hardwoods, mixed hardwoods and conifers (the latter usually hemlock), red pine, white pine, and spruce-hardwoods type from the White Mountains section of New Hampshire.

The results of the comparisons on this basis were practically negative, there being no consistent difference, with the exception of the spruce-hardwoods type from New Hampshire, which gave a somewhat lower factor for the "F" and "H" materials. This was not wholly unexpected considering the difference in latitude and elevation between Connecticut and the locality sampled in New Hampshire. Furthermore, the material came from forests which for the most part are older and have been subject to less disturbance in the past than have those of Connecticut. The organic horizons present consequently are thicker; they have been subjected to decomposition for a longer period of time; and probably contain a larger proportion of the more resistant lignins, cutins, tannins, etc.

CONCLUSIONS

The results of this study together with those of other investigators indicate quite definitely that the carbon content of carbohydrate-rich materials such as forest litter and peat is less than 58 per cent and therefore the conversion factor 1.724 is too low. Although no work relative to this problem has been done at this station on peats, from the results obtained by Gortner (3) and Robinson, McLean, and Williams (6) it would appear that a factor of about 1.86 is approximately correct.

In the case of the organic layers of forest soil, the stage of decomposition cannot be ignored. For the freshly fallen leaves which have not undergone decomposition to any extent 1.89 is very nearly correct; for the decomposing fermentation or duff layers 1.85; and for the well-decomposed, structureless humus, 1.80.

These factors are equally applicable to all the common forest types such as hardwoods, conifers, and hardwood-conifer mixtures found in New England. When the work of investigators in other parts of the country is considered it would appear that the factors recommended in the foregoing may be of more or less universal application.

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THE EFFECT OF VARIOUS SOURCES OF ORGANIC MATTER ON THE PROPERTIES OF SOILS AS DETERMINED BY PHYSICAL MEASUREMENTS AND PLANT GROWTH[1]

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The advent of the internal combustion engine and its substitution for animal power on farms and in cities has greatly reduced the quantity of manure available for use in growing plants. This reduction is particularly marked in regions adjacent to centers of population, where large quantities of manure are utilized for truck gardening, lawns, flower beds, home gardens, parks, and golf courses. The increasing uncertainty as to the quantity and quality of manure still obtainable has stimulated search for satisfactory substitutes.

Users of these substitutes have come to realize that peats, mucks, spent mushroom soil, etc., are not identical with manure in their effect on soils. Part of this difference is undoubtedly due to the relative quantities of avilable nutrients carried by the various materials, but a further part is the result of differences in physical characteristics. Inasmuch as nutrients may be supplied to plants quite as efficiently in commercial fertilizers as in manure, it is important that information be obtained as to the relative effectiveness of various types of organic matter in producing a desirable physical condition of the soil.

Various workers (1, 4, 5, 7) in America and in Europe have called attention to the relation between organic matter in soils and such physical properties as the hygroscopic coefficient, maximum water holding capacity, available water, porosity, and tilth. Certain writers have expressed the opinion that the physical condition of a soil may influence its productivity quite as much as the chemical composition. Changes in moisture and air relationships in the soil not only modify plant growth directly, but they also greatly modify the chemical and biological conditions in the soil and thus affect the plant indirectly.

EXPERIMENTAL METHODS

A greenhouse experiment was conducted during the winter of 1929–30 to determine the relative value of several types of organic matter in modifying

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soils for the growth of bent grass. Five types or sources of organic matter were used: well-rotted stable manure; spent mushroom soil from mushroom houses in Pennsylvania; raw peat from Capac, Michigan; cultivated peat from Newton, New Jersey, and granulated peat moss from Europe. Each of these materials is available in considerable quantities and the quality is fairly uniform. Three soils were selected: a loam and a clay loam of the Sassafras series, and a sandy soil made by mixing 3 parts of sand with 1 part of loam soil.

pH values of the organic materials and soils were obtained by means of a potentiometer with a quinhydrone electrode; and loss on ignition was employed to determine the quantity of organic matter present. Calcium and magnesium were determined from ignited material, the calcium being obtained as calcium oxalate, which was dissolved with sulfuric acid and the resulting oxalic acid titrated with standard potassium permanganate. Magnesium was weighed as magnesium pyrophosphate.

A number of physical constants were used to measure differences in the characteristics of the various organic materials separately, and in mixtures with soil. Hygroscopic coefficients were obtained by exposing air-dry soils in a saturated atmosphere, followed by drying. The available water holding capacity was determined by subtracting the wilting coefficient [indirectly obtained from the hygroscopic coefficient (2)] from the maximum water holding capacity. To determine this latter constant, a part of the soil or material was first saturated, and then sampled after allowing the gravitational water to drain off. Maximum water holding capacities determined in this manner are lower than those found with the Hilgard cup, but the data are regarded as more nearly approaching field water capacities. The rate of percolation of water through each soil was determined by adding water to a hollow cylinder 4 inches in diameter, placed upright with one end inserted into the soil. Sufficient water was added to maintain a depth of at least one-fourth inch above the soil surface, and the quantity collected from the drain in the bottom of the pot in a given period of time furnished the index desired. Pore space was calculated from samples taken with the special tube described by Lebedeff (6).

Mixtures were made of each of the three soils with the five sources of organic matter, at a light and a heavy rate. For the light rate, 73.5 gm. (dry basis) of each type of organic matter were thoroughly mixed with 3,900 gm. of moisture-free soil; and for the heavy rate, 147 gm. of organic matter were added to 3,900 gm. of soil. These quantities are approximately equivalent to 40 and 80 tons, respectively, of material containing 50 per cent moisture applied to an acre of land $6\frac{2}{3}$ inches deep. Each mixture was made in duplicate, thus requiring 20 pots for each soil class, in addition to 2 pots of the untreated soil. Nine-inch porous clay flower pots were used, which were coated on the inside with black asphalt paint to prevent undue evaporation through the walls. Determinations of hygroscopic coefficients, maximum water holding capacity, and loss on ignition were made for each soil mixture at the beginning of the experiment.

After the soil had been firmly packed in, South German mixed bent grass seed was planted on November 18, at the rate of 1 gm. of seed to each pot. The soils were thoroughly moistened at the time of seeding, and thereafter received about 370 cc. of water weekly, the equivalent of 2 inches of rain monthly. For the most part, water was added twice each week, but whenever transpiration was excessive and the grass appeared to be suffering from drouth, an additional supply was provided uniformly for the entire series. Sporadic outbreaks of "brown patch" caused by *Rhizoctonia* spp. were controlled by sprinkling with a dilute solution of mercuric chloride.

Records were kept of atmospheric conditions in the greenhouse during the period covered by the experiment. The mean temperature for the entire 19 weeks was 66°F. This is 6° lower than mean temperatures in the field for a period of equal length from May to September in 1929. The extremes, however, were much greater in the field than in the greenhouse, and the greenhouse temperatures may be regarded as being favorable for grass growth. Total water loss in the greenhouse as measured by an atmometer averaged 105 cc. a week, whereas in the field during the summer of 1929 it averaged 171 cc. The reduced evaporating power of the air in the greenhouse and the uniform supply of moisture largely prevented any serious injury through lack of water, even though the quantity added was comparatively light.

Since the experiment was designed to reveal only the physical effects on the soil of the several types of organic matter, sulfate of ammonia was added in liberal quantities to meet the nutrient requirements of the crop. One-half gram of this fertilizer was supplied in solution to each pot once a month. In turf culture, this quantity has been found ample for maintaining vigorous growth of grass.

At the end of 19 weeks the grass tops were harvested and dry weights obtained. The rate of percolation of water through each soil was then determined. The soils were again sampled to determine their maximum water holding capacities, followed by further sampling to obtain data on pore space. After sieving to remove grass roots, each soil was tested to determine the hygroscopic coefficient and the loss on ignition.

CHARACTERISTICS OF THE MATERIALS USED

The soils included in the experiment were mildly acid in reaction, and the organic content as measured by loss on ignition was medium for the loam and clay loam (table 1). The available water holding capacity was 17.4 per cent for the sandy soil, 32.7 per cent for the loam, and 25.7 per cent for the clay loam. The comparatively low value of the clay loam may be attributed to its poor tilth.

The types of organic matter varied greatly in their physical properties. The manure which had been allowed to decay for one year, was dark brown and easily broken into fragments when partially dried. The spent mushroom soil obtained from mushroom beds near Philadelphia, was originally made by

alternating layers of strawy manures and loam or clay loam.   Upon removal from the beds, the rotted manure was thoroughly mixed with the layers of soil, in which form it was used in these experiments.   The organic matter of this mixture had been more completely transformed in appearance than the well-rotted manure described in the foregoing.   The imported peat moss was light brown and very fibrous, but no large lumps were present.   The raw peat from Michigan was dark brown and coarsely fibrous.   The cultivated peat from New Jersey had lost its fiber, become finely granular, and dark brown to black as a result of the tillage.

The peats and peat moss were strongly acid in reaction, whereas the spent mushroom soil was nearly neutral and the manure distinctly alkaline.   Loss

TABLE 1

Characteristics of the materials used in the tests

MATERIALS AND SOURCES	ACIDITY VALUES	TOTAL OXIDES OF CALCIUM AND MAGNE- SIUM	LOSS ON IGNITION	HYGRO SCOPIC COEFFI- CIENT	MAXI- MUM WATER HOLDING CAPACITY	AVAILA- BLE WATER HOLDING CAPAC- ITY*
	pH	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Types of organic matter:						
Cultivated peat, Newton, New Jersey	5 05	3 47	87 8	51 0	267 6	192 6
Raw peat, Capac, Michigan	4 03	0 80	90 1	39 9	346 4	287 7
Imported peat moss, Europe	4 42	0 34	97 1	54 6	1,034 9	954 6
Spent mushroom soil, Pennsylvania	6 65	1 56	23 9	25 1	70 5	33 6
Well-rotted stable manure, New Jersey	8 50	2 38	68 9	76 7	288 3	175 4
Soil classes:						
Sandy soil	5 80	..	2 6	2 2	20 6	17 4
Loam	6 10		6 8	3 9	38 4	32 7
Clay loam	5 93		6 5	7 2	36 2	25 7

* Available water holding capacity determined by subtracting the wilting coefficient (indirectly determined from hygroscopic coefficient) from the maximum water holding capacity.

on ignition data indicate a far higher organic matter content for the three peats than for manure, whereas the mushroom soil contained only about one-third as much.   The hygroscopic coefficients of the organic materials were not proportional to their organic content.   Thus, the coefficient of New Jersey peat was 11.1 per cent greater than that of Michigan peat, although the organic content of the latter was slightly higher.

The maximum water holding capacities of the peats compared very favorably with manure, but spent mushroom soil showed a comparatively low capacity. The imported peat moss possessed the greatest capacity for total water, and also for water available to plants.   This material, however, absorbed water very slowly.   In order to insure complete saturation of the particles, it was

necessary to plug the bottom of the flower pot containing the peat moss, add an excess of water and allow it to stand for 24 hours. This property was not apparent in New Jersey peat, unless it had been first thoroughly air-dried. In agricultural practice, the slow water absorption of dry peat may cause trouble when the material is mixed with soil in droughty periods. Layers of peat placed below the surface of the soil may also be harmful for the same reason if they are thoroughly dry when laid down, or become dry later on.

EXPERIMENTAL RESULTS ON SANDY SOIL

Sandy soils are notoriously low in water holding capacity, and are frequently so open that water passes through rapidly without greatly benefiting plant growth. The admixture of organic matter or clay to such soils is usually recommended to correct their deficiencies. In these investigations, the addition of the peaty materials, spent mushroom soil, or manure increased the capacity to retain available water, when this property was measured after grass had been grown on the soil mixtures for 19 weeks (table 2). The imported peat proved most effective in this respect, but considerable difficulty was experienced at the beginning of the experiment in keeping the soil mixture containing this material sufficiently moist to germinate seed.

The data indicate that some time is required before the full effectiveness of organic materials in raising the water holding capacity is attained; and for certain materials, such as manure and peat moss, this increase in effectiveness is more than offset by their rapid loss through decomposition. From the practical standpoint, it is noteworthy that the peats have been more valuable for increasing moisture capacity than has well-rotted manure. Although spent mushroom soil improved the water relations of the soil, it was not so effective as manure for the period covered by the experiment. It is also apparent from the data that doubling the quantity of organic matter raised the water holding capacity of the soil in every case.

Cultivated New Jersey peat, raw Michigan peat, and spent mushroom soil reduced the pore space of sandy soils; however, large quantities were less efficient than smaller additions. Manure proved less effective in reducing pore space than did the aforementioned materials, and peat moss was in turn less effective than manure since it actually increased total pore space when used at the heavier rate.

Percolation rates were roughly correlated with pore space of the soil mixtures. Cultivated New Jersey peat reduced percolation to a lower value than any other material. Spent mushroom soil and raw Michigan peat also reduced percolation considerably below that of the untreated soil. Light applications of peat moss or manure likewise lowered the rate of percolation, but the heavier applications greatly increased it.

Grass yields produced on these soil mixtures may be taken as a measure of the effectiveness with which these organic materials modified the soil for plant growth. In general, these yields indicate that heavy applications were more

valuable than light additions, with all types of organic matter greatly improving the productivity of the sandy soil. Yields were not highly correlated with the increases in available water holding capacities of the various mixtures. Thus, although mixtures containing cultivated New Jersey peat did not average so high in water holding capacity as those containing Michigan peat, the yields

TABLE 2

The effect of different types of organic matter additions on the physical properties of a sandy soil, and on the yields of grass produced

TYPE OF MATERIAL MIXED WITH THE SOIL	AVAILABLE WATER HOLDING CAPACITY*		PORE SPACE OF SOIL AFTER GROWTH OF GRASS		PERCOLATION OF WATER IN 15 MINUTES	GRASS YIELDS PER POT (DRY WEIGHT OF TOPS)
	Before growth of grass	After growth of grass	Actual	Difference from check		
	per cent	*per cent*	*per cent*	*per cent*	*cc*	*gm*
A Light additions of organic matter—73 5 gm dry matter to 3,900 gm moisture free soil						
Cultivated New Jersey peat	14 9	18 1	47 1	−18 2	286	9 3
Raw Michigan Peat	17 6	20 0	50 5	−12 3	469	8 7
Imported peat moss	24 2	24 7	56 9	−1 2	678	9 4
Spent mushroom soil	15 5	16 2	49 2	−14 6	417	9 4
Well-rotted manure	20 2	17 1	50 1	−13 0	639	11 5
B Heavy additions of organic matter—147 0 gm dry matter to 3,900 gm moisture-free soil						
Cultivated New Jersey peat	20 1	21 2	50 0	−13 2	298	10 3
Raw Michigan peat	24 6	20 4	53 7	−6 8	535	9 1
Imported peat moss	33 8	30 5	62 9	+9 2	1,102	10 6
Spent mushroom soil	18 5	19 4	52 4	−9 0	436	9 6
Well-rotted manure .	23 8	19 5	55 3	−4 0	1,052	9 3
Average of both light and heavy additions of organic matter						
Cultivated New Jersey peat	17 5	19 6	48 5	−15 8	292	9 8
Raw Michigan peat	21 1	20 2	52 1	−9 5	502	8 9
Imported peat moss	29 0	27 6	59 9	+4 0	890	10 0
Spent mushroom soil	17 0	17 8	50 8	−11 8	426	9 5
Well-rotted manure	22 0	18 3	52 7	−8 5	845	10 4
Check—no organic matter added	17 4	16 1	57 6		767	6 2

* Available water holding capacity determined by subtracting the wilting coefficient (indirectly determined from hygroscopic coefficient) from the maximum water holding capacity

of grass on the former were much higher. Also, New Jersey peat mixtures produced nearly as heavy yields of grass as those containing peat moss, although the moisture retaining power of the peat moss mixtures was far greater.

The explanation for the different effects of the various types of organic matter seems to lie in the modification of the soil structure. Moisture was supplied to the grass in this experiment in periodic waterings once or twice

acid was also determined by titration. After equilibrium was judged to be complete the chloride solutions were added twice more to take care of the low adsorption energy of potassium. All the soils were treated 12 times to replace the sodium with the other base. The soils were then repeatedly washed with water until the soluble chlorides were removed or reduced to such a low concentration that sedimentation aided by centrifuging would not occur rapidly. By washing with water alone, the soils treated with iron and aluminum were completely freed from soluble chlorides. The soils that had been treated with sodium, potassium, ammonium, hydrogen, calcium, and magnesium, after being washed with water were then washed with 95 per cent alcohol. By the alcoholic washings the calcium, magnesium, hydrogen, and ammonium soils were entirely freed from soluble chlorides. The sodium and potassium soils were nearly freed from soluble chlorides by alcoholic washing, judged by the fact that the last wash solution recovered before sedimentation failed to occur contained only several parts of chloride per million.

The soils treated as previously described were not saturated to the same degree with the various bases, as the chloride solutions were not neutralized. Neutralization of ferric and aluminum chloride would precipitate the cation, and to keep the series uniform the other solutions were not neutralized. The degree of saturation therefore would depend on the ratio of the energy of adsorption for the base and hydrogen, and on the amount of hydrogen present as a result of hydrolysis.

In washing the soils free from chlorides there was also opportunity for hydrolysis to take place, replacing some of the base with hydrogen. Analyses of the samples in which the total bases were determined indicated that the soils treated with aluminum chloride had absorbed only a trace of the base. Smaller quantities of the magnesium than of the other bases were adsorbed. Enough base was adsorbed in every case to have a decided effect on the physical characteristics of the soil. The aluminum-treated soil for instance had a noticeably low specific gravity, whereas the iron-treated soil had an intense red color.

DATA COLLECTED

The pH values presented in tables 2 to 9 were determined after the soils had been air-dried for approximately six months. The quinhydrone electrode was used in making these determinations, and the potentials were read about 30 seconds after the soil, water, and quinhydrone had been mixed. The soil-water ratios used were 1:1, 1:2.5, 1:10, and 1:100 and data are presented for these mixtures. A few determinations were made using other ratios and these indicated that the curves obtained were regular. In figure 1 the pH values shown are the average values for the six soils and the curves are drawn from the four points only, as indicated. The results given are reproducible under standard conditions, but there is considerable variation in the figures obtainable as drying progresses. When freshly prepared the soils treated with ferric

chloride did not show a greater degree of acidity at the 1:100 dilution than the hydrogen chloride-treated soils.   The aluminum chloride-treated soils showed great variation in degree of acidity, especially in the greater dilutions.   When

TABLE 2

pH values of HCl-treated soils

SOIL-WATER RATIO	SOIL NUMBERS						
	1	2	6	8	11	12	Average
1:1	2 80	2 80	3 17	2 97	3 33	3 30	3 06
1:2 5	3 15	3 15	3 47	3 27	3 45	3 45	3 32
1:10	3 15	3 25	3 52	3 38	3 47	3 54	3 39
1:100	3 65	3 52	3 82	3.80	3 96	3 75	3 75

TABLE 3

pH values of FeCl$_3$-treated soils

SOIL WATER RATIO	SOIL NUMBERS						
	1	2	6	8	11	12	Average
1:1	2 97	3 03	3 11	3 14	3 18	3 18	3 10
1:2 5	3 36	3 38	3 36	3 41	3 34	3 34	3 36
1:10	3 43	3 41	3 38	3 36	3 36	3 31	3 38
1:100	3 63	3 53	3 58	3 58	3 61	3 61	3 59

TABLE 4

pH values of AlCl$_3$-treated soils

SOIL-WATER RATIO	SOIL NUMBERS						
	1	2	6	8	11	12	Average
1:1	4 16	4 36	4 16	4 23	4 06	4 20	4 19
1:2 5	4 56	4 56	4 16	4 38	4 10	4 40	4 36
1:10	4 36	4 50	4 10	4 50	4 13	4 50	4 34
1:100	4 70	4 80	4 70	4 83	4 53	4 79	4 72

TABLE 5

pH values of NH$_4$Cl-treated soils

RATIO	SOIL NUMBERS						
	1	2	6	8	11	12	Average
1:1	5 63	5 56	5 66	5 61	5 56	5 56	5 59
1·2 5	5 75	5 75	6 01	5 65	5 86	5 82	5 81
1:10	6 42	6 52	6 56	6 22	6 46	5 96	6 36
1:100	6 69	6 77	6 65	6 62	6 82	6 42	6 66

each week.   Soil mixtures which were extremely porous permitted more rapid percolation than soils which were less open.   Surface evaporation from open soils must also have been relatively great.   Therefore, the increased potential ability of the mixtures to retain moisture was not fully utilized when the relatively coarse textured peat moss and raw Michigan peat were incorporated. It seems probable that such conditions may also exist in the field when moisture is supplied in relatively large amounts over a short period of time, as in thundershowers, or periodic heavy waterings.   These results indicate that fine textured materials such as cultivated peat and spent mushroom soil are more suitable substitutes for manure on sandy soils than coarse textured materials such as raw Michigan peat and imported peat moss.

EXPERIMENTAL RESULTS WITH A LOAM SOIL

The data obtained from mixtures of loam soil with each of the organic matter sources, when compared with those from sandy soil, show that the manure substitutes do not effect all soil types similarly (table 3).   The loam used in these tests had a fairly high organic matter content before treatment, and with the system of limited watering followed, one should not expect so marked an effect from additions of organic matter as for sandy soil.   However, the incorporation of every type of organic matter increased the available water holding capacity of the loam.   Imported peat moss was most effective in raising the moisture retaining power, with raw Michigan peat, manure, cultivated New Jersey peat, and spent mushroom soil following in the order named. With the exception of the spent mushroom soil, the ability of the mixtures to retain moisture was greater after the growth of grass than when the organic matter was first incorporated.

Pore space of the loam soil was not greatly changed by the addition of any type of organic matter, except peat moss.   This latter type of material markedly increased the pore space, particularly when used at the heavier rate.   Peat moss was also the only material which notably increased the percolation rate of water.   When used at the lighter rate, all of the materials reduced percolation.   Soil mixtures receiving heavy additions of each material had higher rates of percolation, and in the case of peat moss and Michigan peat, these rates exceeded that for untreated loam.

It is evident from the yields of grass obtained that the open structure produced by heavy additions of peat moss was less desirable for plant growth than a more compact structure.   For all other treatments the grass yields were 13 to 40 per cent greater than on untreated soil.   There was a fairly close correlation between available water holding capacity of the treated soils and the yields of grass produced, if the heavy application of peat moss is disregarded.   Apparently, the addition of organic matter to the soil permitted more complete utilization of the comparatively scanty moisture supply, except where the structure had become exceedingly open as the result of the treatment given.

Cultivated New Jersey peat and raw peat from Michigan were about equally valuable for improving the loam soil for plant growth, and both were moderately satisfactory as substitutes for manure.   Peat moss seems to be valuable for modifying the physical condition of this soil, only when used in moderate quantities.   Under conditions of abundant moisture, peat moss may possibly be

TABLE 3

The effect of different types of organic matter additions on the physical properties of a loam soil, and on the yields of grass produced

TYPE OF MATERIAL MIXED WITH THE SOIL	AVAILABLE WATER HOLDING CAPACITY*		PORE SPACE OF SOIL AFTER GROWTH OF GRASS		PERCOLA-TION OF WATER IN 30 MINUTES	GRASS YIELDS PER POT (DRY WEIGHT OF TOPS)
	Before growth of grass	After growth of grass	Actual	Differ-ence from check		
	per cent	*per cent*	*per cent*	*per cent*	*cc*	*gm*
A Light additions of organic matter—73 5 gm dry matter to 3,900 gm moisture free soil						
Cultivated New Jersey peat	33 2	35 8	59 5	−5 0	354	12 9
Raw Michigan peat	35 6	35 6	61 1	−2 4	458	12 0
Imported peat moss	37 2	38 9	65 5	+4 6	592	13 5
Spent mushroom soil	33 1	33 4	62 7	+0 2	491	13 3
Well-rotted manure	33 9	35 1	62 9	+0 5	472	13 0
B Heavy additions of organic matter—147 0 gm dry matter to 3,900 gm moisture free soil						
Cultivated New Jersey peat	32 6	33 5	63 9	+2 1	595	12 7
Raw Michigan peat	37 5	39 0	66 3	+5 9	800	13 4
Imported peat moss	42 1	46 5	69 9	+11 7	1,235	9 9
Spent mushroom soil	32 7	31 2	61 0	−2 6	485	12 2
Well rotted manure	34 0	35 1	63 8	+1 9	595	14 8
Average of both light and heavy additions of organic matter						
Cultivated New Jersey peat	32 9	34 6	61 7	−1 4	474	12 8
Raw Michigan peat	36 5	37 3	63 7	+1 8	629	12 7
Imported peat moss	39 6	42 7	67 7	+8 1	913	11 7
Spent mushroom soil	32 9	32 3	61 8	−1 4	488	12 7
Well-rotted manure	33 9	35 1	63 3	+1 1	533	13 9
Check—no organic matter added	32 8	31 0	62 6	.	682	10 6

* Available water holding capacity determining by subtracting the wilting coefficient (indirectly determined from the hygroscopic coefficient) from the maximum water holding capacity

useful in heavier amounts.   Apparently less physical improvement is produced by the incorporation of spent mushroom soil in liberal quantities than from the use of manure, cultivated peat, or raw Michigan peat.   This behavior may be attributed to its comparatively low content of organic matter and high content of silt and clay.

EXPERIMENTAL RESULTS WITH A CLAY LOAM SOIL

The addition of organic matter of any type improved the water holding capacity of the clay loam soil; and further, heavy additions were more effective than light ones in this respect (table 4). Spent mushroom soil was least effective in bringing about this change whereas imported peat moss proved most

TABLE 4

The effect of different types of organic matter additions on the physical properties of a clay loam soil, and on yields of grass produced

TYPE OF MATERIAL ADDED TO THE SOIL	AVAILABLE WATER HOLDING CAPACITY*		PORE SPACE OF SOILS AFTER GROWTH OF GRASS		PERCOLA-TION OF WATER IN 30 MINUTES	GRASS YIELDS PER POT (DRY WEIGHT OF TOPS)
	Before growth of grass	After growth of grass	Actual	Differ-ence from check		
	per cent	*per cent*	*per cent*	*per cent*	*cc*	*gm*
A Light additions of organic matter—73 5 gm dry matter to 3,900 gm moisture-free soil						
Cultivated New Jersey peat	29 0	28 0	60 0	+0 7	333	11 3
Raw Michigan peat	31 5	30 3	62 8	+5 4	227	11 3
Imported peat moss	36 8	33 3	64 6	+8 4	896	11 4
Spent mushroom soil	30 7	27 3	60 1	+0 8	287	11 3
Well-rotted manure	32 1	29 8	61 4	+3 0	232	11 5
B Heavy additions of organic matter—147 0 gm dry matter to 3,900 gm moisture-free soil						
Cultivated New Jersey peat	31 0	30 1	62 7	+5 2	515	10 8
Raw Michigan peat	35 9	33 4	64 9	+8 9	805	10 9
Imported peat moss	39 9	35 0	69 0	+15 8	1,562	10 3
Spent mushroom soil	30 8	28 0	62 2	+4 4	290	12 0
Well rotted manure	31 1	32 9	64 1	+7 6	885	11 7
Average of both light and heavy additions of organic matter						
Cultivated New Jersey peat	30 0	29 0	61 3	+2 9	424	11 0
Raw Michigan peat	33 7	31 8	63 8	+7 0	516	11 1
Imported peat moss	38 3	34 1	66 8	−12 0	1,229	10 8
Spent mushroom soil	30 7	27 6	61 1	+2 5	288	11 6
Well-rotted manure	31 6	31 3	62 7	+5 2	558	11 6
Check—no organic matter added	26 7	26 0	59 6		320	9 6

* Available water holding capacity determined by subtracting the wilting coefficient (indirectly determined from the hygroscopic coefficient) from the maximum water holding capacity

effective. Cultivated New Jersey peat was slightly less efficient, and raw Michigan peat somewhat more efficient than manure in raising the moisture retaining power of the clay loam.

Spent mushroom soil likewise produced the least change in pore space, although each of the materials increased this property. Peat moss additions

caused the greatest modification, raising the percentage from 59.6 for untreated soil to 69.0 when heavy additions were made. Cultivated peat was less effective than manure whereas raw Michigan peat was more effective in raising the percentage of pore space.

Percolation rate for the untreated clay loam was not so low as might be expected, largely because numerous cracks developed under the system of light watering, and water added to the surface passed downward through these channels without actually moving through the body of the soil itself. The addition of organic matter in any form, except spent mushroom soil, prevented the formation of cracks in the soil mass and also produced an appreciable increase in the rate of percolation. In the case of manure and the three types of peat, heavy applications brought percolation rates to a higher point than light additions. It is noteworthy that the effectiveness of the five types of materials compared varied, with their texture. Spent mushroom soil being largely composed of silt and clay produced the least change, and the granular cultivated peat had considerably less influence than the fibrous peat from Michigan. The large increase in percolation rate which resulted from the use of peat moss is associated with its fibrous condition and bulky nature.

From a practical standpoint it is apparent that the incorporation of very large quantities of peat moss under conditions of limited moisture supply may produce an undesirable medium for plant growth, because of the increase in porosity and percolation, and the very slow absorption of water by the peat moss when it has become air-dry. On the other hand, it is evident that organic materials of a fibrous nature are more effective in improving the physical condition of a heavy clay loam than those which are largely non-fibrous. It seems likely that this difference would be even more noticeable under conditions of excessive moisture supply which tend to make the soil more compact.

The yields of grass produced on the clay loam mixtures were in every case considerably higher than for untreated clay loam. The productivity of the mixtures was not closely associated with the changes produced in their physical properties. Thus, imported peat moss produced the lowest yields, even though it was most effective in changing the physical nature of the soil. Spent mushroom soil modified the soil least, but produced as large yields of grass as manure. Cultivated New Jersey peat proved about as efficient as raw Michigan peat in stimulating plant growth, both being nearly as effective as manure.

The relative value of each manure substitute on heavy soils cannot be determined from these data alone. A similar experiment conducted under conditions of abundant moisture supply should supplement these findings and permit the formulation of more definite conclusions. These data taken alone seem to indicate that additions of organic matter to clay loams of this type are beneficial to plant growth and that materials which are moderately fibrous are satisfactory substitutes for manure. However, the addition of organic matter of any type seems desirable; the question being one of the relative effectiveness of the various types.

THE PERSISTENCE OF ORGANIC MATTER

One of the important considerations in choosing a source of organic matter for the modification of any soil, is the length of time the material will persist and exert its influence.   To obtain this information for each type of organic matter under test, data were obtained for loss on ignition of the soil mixtures at the beginning of the experiment and again after grass had been grown on the soils for 19 weeks.   These data (table 5) show that the persistence varies with soil type, losses being about three times as great in the sandy soil as in the clay loam.   For any one soil, however, considerable difference in rapidity of decomposition was observed between the various types of organic matter.

When data from all three soils were averaged, 17.4 per cent of the native organic matter present was found to have been lost during the period of 19 weeks when grass was grown on the untreated soil.   Soil mixtures containing mush-

TABLE 5

Persistence of organic matter mixed with soil and cropped to grass, as measured by loss on ignition before and after cropping (147 gm dry matter to 3,900 gm moisture-free soil)

SOURCE OF ORGANIC MATTER ADDED	SANDY SOIL			LOAM			CLAY LOAM			AVERAGE PERCENTAGE LOSS FOR ALL THREE SOILS
	Before growth	After growth	Difference	Before growth	After growth	Difference	Before growth	After growth	Difference	
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Cultivated New Jersey peat	5 0	4 3	0 7	9 6	8 6	1 0	9 4	8 9	0 5	9 9
Raw Michigan peat	7 1	4 4	2 7	9 2	8 8	0 4	9 9	8 5	1 4	18 8
Imported peat moss	6 2	3 4	2 8	10 8	9 0	1 8	10 4	8 3	2 1	27 3
Spent mushroom soil	3 6	2 3	1 3	7 5	7 3	0 2	7 2	7 1	0 1	13 4
Well rotted manure	5 6	2 8	2 8	9 7	7 7	2 0	9 0	6 6	2 4	32 4
Control (no organic matter added)	2 6	1 6	1 0	6 8	6 3	0 5	6 5	6 1	0 4	17 4

room soil lost 13.4 per cent, whereas those containing cultivated peat lost only 9.9 per cent.   Soil containing raw Michigan peat lost 18.8 per cent, and imported peat moss 27.3 per cent, but even these amounts were less than that noted for manure, 32.4 per cent.

It is dangerous to draw final conclusions from data collected under such a limited range of conditions, but the various substitutes for manure seem to have been somewhat more persistent in the soil than manure itself, and may reasonably be expected to effect the soil to which they have been added fully as long as manure if not to the same degree.   Cultivated peat will doubtless prove more persistent than raw peat or peat moss, since it has already undergone considerable decomposition under tillage and probably contains a higher percentage of materials which are resistant to decay.

SUMMARY AND CONCLUSIONS

A greenhouse experiment was conducted to determine the relative effectiveness of five types of organic matter for improving the physical condition of soil for plant growth. Each type of organic matter was added at a light and heavy rate to a sandy soil, a loam, and a clay loam. Certain physical properties of the untreated soils and soil mixtures were measured when the organic matter was first incorporated, and again after German mixed bent grass had been grown for 19 weeks.

Under the system of limited watering and adequate fertilization followed, sandy soil was improved for growth of grass by the addition of all types of organic matter. An analysis of the data on physical properties of the soil mixtures indicates that cultivated New Jersey peat and spent mushroom soil are the most satisfactory types of material for sandy soil, followed in order by raw Michigan peat, manure, and peat moss. Peat moss mixtures with sandy soil were relatively undesirable because of the slowness with which the dry material absorbed water, and the very open structure produced.

The addition of all types of organic matter also improved the loam soil for plant growth, except where large amounts of peat moss were used. The most effective substitutes for manure were cultivated New Jersey peat and raw Michigan peat. Peat moss was satisfactory only when used at the light rate; and spent mushroom soil was the least effective of the organic materials tested, probably because of a relatively high content of silt and clay.

The untreated clay loam soil had a compact structure, and cracked badly in this experiment. The addition of organic matter of any type largely prevented the formation of cracks, modified the physical properties of the soil and increased plant growth. From a consideration of the physical properties of the treated soils and the grass yields produced, fibrous organic materials such as manure and raw Michigan peat seem to be somewhat more suitable amendments than non-fibrous cultivated peat or mushroom soil. The use of peat moss in large quantities with the system of limited watering used, seems undesirable because of the slow absorption of water by the dry material and the exceedingly open structure produced.

Peaty materials which have become thoroughly dry take up moisture very slowly and may produce an undesirable physical condition when incorporated with the soil, particularly if the moisture supply is limited or if water is supplied infrequently in large amounts. The undesirable effect may be intensified in the field if layers of dry peat are placed below the surface of the soil within the root zone, or if the peat layers so placed become very dry.

· Loss on ignition determinations made at the beginning and end of the experiment for all treated soils, indicate that the organic matter of manure is more rapidly decomposed and lost than that of any other type of organic matter tested. The organic matter of cultivated peat and mushroom soil was more persistent than that of raw peat or peat moss, probably because the former sub-

stances had already undergone considerable decomposition and contained a higher percentage of compounds resistant to decay.

It is clearly shown that other types of organic matter may be substituted satisfactorily for barnyard manure, if the nutrient deficiencies are corrected by the addition of fertilizer.   The choice of the most desirable type of organic matter depends on the properties of the material itself, and the nature of the soil with which it is mixed.

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PLATE 1

THE EFFECT ON PLANT GROWTH OF INCORPORATING VARIOUS TYPES OF ORGANIC MATTER
WITH THE SOIL

Fig. 1. Relative values of the three untreated soils used in the experiment, as indicated by plant growth

Pot	Soil class	Yield of grass tops gm.
31	Sandy soil...	. 6 2
32	Loam	10 6
33	Clay loam....	9 6

Fig. 2. The effects on plant growth of adding various sources of organic matter to a sandy soil (147 gm. dry matter to 3,900 gm. moisture-free soil)

Pot no.	Material added	Yield of grass tops gm.
6	New Jersey peat.	10 3
7	Michigan peat	9 1
8	Imported peat moss	10 6
9	Spent mushroom soil	9 6
10	Well rotted manure	9 3
31	Control (No organic matter added)	6 2

Fig 3. The effects on plant growth of adding various sources of organic matter to a clay loam soil (147 gm. dry matter to 3,900 gm. moisture-free soil)

Pots removed from soil. Observe cracks formed where no organic matter was added and where spent mushroom soil was added. White line indicates level of the soil.

Pot no.	Material added	Yield of grass tops gm.
26	New Jersey peat	10 8
27	Michigan peat	10.9
28	Imported peat moss	10 3
29	Spent mushroom soil......	12.0
30	Well rotted manure	11 7
33	Control (No organic matter added)...	9.4

FIG. 1

FIG. 2

EXCHANGEABLE CATIONS OF THE SOIL AND THE PLANT: I. RELATION OF PLANT TO CERTAIN CATIONS FULLY SATURATING THE SOIL EXCHANGE CAPACITY

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Considerable time and attention are at present paid to questions connected with the condition of the soil which depends upon the composition of its exchangeable cations and with the explanation of the relation of this composition to the chemical, physical, and physico-chemical properties of the soil. However, the relation of the growth of plants and the life and activities of microorganisms to the composition of the exchanged bases in the soil, problems that have no less theoretical interest and at the same time are of considerable practical importance, have received up until the present time, almost no experimental study. Experiments with plants and microörganisms along this line may give us a whole series of very interesting conclusions which can find application to agriculture. Plants and microörganisms form a very sensitive indicator of the changes that take place in soil as a result of a change of its properties and, therefore, also of its exchanged bases. The application of this biological method of investigation of various questions related to the absorbing properties of the soil in the broadest possible sense should not be less fertile in results than the application of chemical and physico-chemical methods. It is quite clear that the broad utilization of this method of investigation together with others that have already become established in soil science is very desirable both in the interest of soil science.and agriculture. Our knowledge will thereby become broadened and completed. As an example of the possibility of application of the biological method in soil science, let me point out the question which has interested soil scientists for a long time, namely, can the soil contain exchangeable hydrogen or is the cause of soil acidity always the exchangeable aluminum. As a matter of fact, this question can be answered very easily and simply by the plant: the relation of the plant to soil, treated with aluminum salt until all the exchangeable bases have been replaced, on the one hand, and treated with 0.05 N HCl also until all the replaceable bases have been removed, on the other, prove to be, after liming of both, totally different; one cannot say that we are dealing in both cases with exchanged aluminum, and the action of lime upon soil treated with 0.05 N HCl solution can be explained only by the presence in it of exchangeable hydrogen.

The almost total absence of experiments with plants grown in soils artificially saturated with one base or another is explained, of course, by the complexity and comparatively high cost of preparation of soil material needed for such experiments, and the need of certain special facilities for carrying out such experiments.

In my case, for example, the investigations of the biological methods of the questions connected with the absorbing properties of soil, begun in 1911 (3), could bring them to a convenient state only now, the problem outlined 20 years ago not being completed as yet.

Before coming to the fundamental subject of this article, namely, the question of the relation of plants to various cations when each is completely saturating the total exchange capacity of a given soil, I will first consider the marked difference in importance for plant growth of various nutritive exchangeable bases, as brought out in my experiments.

It is known that among the nutritive elements essential for the life of plants, only three—Ca, Mg, and K— are found (or may be found) in the soil in an exchangeable condition; we know further that whatever the quantity of these cations in the soil in an exchangeable condition, these cations are also always found in the soil in a condition not capable of exchange; whereby there is usually present in soil considerably less unexchangeable Ca than exchangeable. (The cultivated horizon of the tchernoziem, with which the first vegetation experiments here described were carried out, contained 1.26 per cent exchangeable CaO and 0.5 per cent unexchangeable.) An inverse relationship is found in the case of Mg and K. (The above horizon contained 0.17 per cent exchangeable and 0.93 per cent unexchangeable and MgO 0.07 per cent exchangeable and 2.04 per cent unexchangeable K_2O.) Soils contain, as a rule, comparatively little exchangeable magnesium, but still a very definite quantity; no soil has been found as yet which has contained no exchangeable magnesium. But this is not true in the case of potassium; the ordinary concentration of exchangeable potassium in soil is a few hundredths of a per cent of K_2O or about 1 mgm. equivalent in 100 gm. of soil. This is such an insignificant quantity that, considering the complexity of replacement of potassium from soil, the complexity of the very method of determination of the quantity of potassium, and, especially the possibility of the presence of traces of potassium in the reagents used, which can give, in the case of the large quantities of reagent used in the determination of exchanged potassium. amounts of potassium of the same order, one should not treat this quantity obtained as one really present in soil. Therefore, until a more sensitive method of determination of exchangeable potassium is developed (this is true, of course, also for the exchanged magnesium and in general for exchanged cations when they are present in soil in very small concentrations) our ideas of its concentration in soil will be more in the nature of guesses.

Why is there then so little exchangeable magnesium and potassium in soil, when the total concentration of these elements in soil is so large? One can

answer this question only in a very general way. It is possible, for example, that as a result of the presence, now or in former times, in the soils of considerable quantities of $CaCO_3$, all the bases present (or which were present) on the surface of the primary particles of soil were replaced by calcium and we could thus not have exchanged potassium (or magnesium), if there were not formed, during the processes of further soil formation, new surfaces accessible to other replacing cations. There is no doubt that such surfaces are being formed and that the inner layers of soil particles may become outer. Such a surface, newly formed, may of course contain, depending on its mineralogical composition, exchangeable potassium and magnesium. It is understood that such a process of new formation of the outer surface must be very slow and, therefore, the quantity of exchangeable potassium and magnesium (and sodium) found in the soil at any given moment may be very little.

Of course, these newly forming outer surfaces must also contain exchangeable cations of calcium, but since the soil contains less unexchangeable calcium than unexchangeable magnesium and potassium, it is natural that, on the average, there will be less exchangeable calcium than exchangeable potassium and magnesium.

Another explanation of this phenomenon is possible. There is no doubt that all soil particles independent of their size, possess exchange capacity, but the intensity of exchange, being directly dependent on the degree of dispersion of the substance in a unit of its volume, will be considerably greater in a colloidal fraction than in a non-colloidal one. If we call the exchange of a colloidal fraction "intensive exchange," we may say that the non-colloidal part of the soil also possesses exchange capacity, but only "extensive." An exchange of a similar order (quantitatively) must also take place in the colloidal soil fraction in respect to the small *residual* quantities of exchangeable cations present, and also in the cations of the newly forming exterior surfaces. The bases calcium, magnesium, and potassium (and sodium) must be on the surface of these larger soil particles; but since the intensely exchangeable calcium forms a large part of the total calcium content of the soil, and the exchangeable magnesium and potassium (and sodium) only a minute part of the total concentration, it is natural to expect on the surface of the larger soil particles relatively more of the exchangeable bases magnesium and potassium than of calcium. At least, there is no doubt that the replacement of exchangeable bases from the soil by treating it for a certain period of time with a salt solution of any desirable cation is never brought to completion. We are replacing only the intensively exchangeable bases and a part of the extensively exchangeable; however, a larger or smaller part of the exchangeable cations of the large fractions plus the exchangeable bases on the newly formed outer surfaces of the soil particles, i.e. in general, the extensively exchangeable cations, remain in the soil. Their total replacement is, probably, a process very prolonged. One may speak of the replacement of exchangeable bases from the soil as only *practically* complete for a given purpose; thus, for example, for the comparative

study of the content of exchangeable bases in soil, it is sufficient at the present time to determine the quantities of exchangeable calcium and magnesium, which are practically replaced from the soil by the given methods, but these methods are still inadequate for the study of phenomena connected with exchangeable potassium. In regard to questions of plant nutrition, we must be able to determine in the soil the quantities of extensively exchangeable bases of calcium and magnesium as well as potassium.

Whatever the causes of the aforementioned phenomenon, however, the very facts of the low content of exchangeable potassium and magnesium in soil, both in absolute and relative concentrations, and just the reverse relation for calcium remain: calcium is present, as an exchangeable base, in the soil in a special position when compared with the other exchangeable soil bases. We will soon see that plants also point to the special position occupied by calcium among the other exchangeable bases necessary for plant life.

As I have mentioned in one of my previous papers (1), I began in 1912 a large vegetation experiment using the cultivated horizon of a tchernoziem rich in humus, artificially saturated completely with calcium, according to my method. The purpose of the experiment was to obtain a soil which reacts strongly to potassium fertilization (natural soils usually react in pot experiments very weakly to potassium, even when large amounts of nitrogen and phosphorus fertilizers are employed), and thus make it possible to study the problems of potassium nutrition by plants not only in water and in sand cultures, but also in soils. The soil thus prepared, containing calcium practically alone of the exchangeable bases, was used for a vegetation experiment carried out in pots (20 by 20 cm.); the same basic fertilizer (0.2 gm. of nitrogen as $NaNO_3$ and 0.2 gm. phosphoric acid as NaH_2PO_4) and varying quantities of potassium sulfate (from 0.05 to 1.0 gm. K_2O per pot) were added. The experiment was carried out with three kinds of plants: oats, mustard, and buckwheat.

All the combinations tested gave for each plant, within the limits of error of pot experimentation, exactly the same yields. The experiment did not yield the results expected. The soil even saturated with calcium did not react to potassium, with the given quantities of nitrogen and phosphorus. This fact alone, however, represents a great interest in connection with questions concerning the rôle of exchangeable nutritive bases in the growth of plants. We will, therefore, consider this experiment and, since, as has been shown, all the pots containing the same plant gave similar yields, we will not report all the extensive data obtained in this experiment, but will limit ourselves to those shown in table 1.

The results show that both the original tchernoziem and the tchernoziem saturated only with calcium had no need of potassium for any of the three plants; since the basic fértilizer did not contain any magnesium and since the original tchernoziem did not need it, the results of the experiment show that the tchernoziem saturated with calcium did not need any magnesium as well.

TABLE 1

Results of vegetative experiment in a tchernoziem saturated with calcium

Crop yield in grams per pot   Basic fertilizer, NaNO₃ and NaH₂PO₄

GROUP	SOIL	NO POTASSIUM			0.05 GM K₂O			0.1 GM K₂O			0.15 GM K₂O			0.20 GM K₂O			0.30 GM K₂O		
		Grain	Straw	Total	Grain	Straw	Total	Grain	Straw	Total	Grain	Straw	Total	Grain	Straw	Total	Grain	Straw	Total
Oats	Original soil	10.2	10.8	21.0	10.4	10.6	21.0	9.8	11.0	20.8	9.7	11.0	20.7			20.6	....	....	....
	Soil saturated with calcium	9.3	11.1	20.4							9.6	10.3	19.9	10.0	10.6	20.6	9.9	10.9	20.8
Mustard	Original soil	5.4	13.2	18.6							5.5	13.4	18.9				....	....	....
	Soil saturated with calcium	5.4	13.0	18.4	5.6	12.8	18.4	5.3	12.9	18.2	5.2	12.1	17.3	5.5	12.6	18.1	5.6	12.7	18.3
Buckwheat	Original soil	13.2	12.6	25.8				12.2	13.1	25.3				13.6	12.4	26.0	..	..	..
	Soil saturated with calcium	13.1	12.0	25.1										13.4	12.7	26.1	13.1	12.7	25.8

Similar results were obtained in the experiment, described in the following, carried out in 1929, on the relation of magnesium in a tchernoziem saturated with hydrogen; the same yield of oats was obtained in this experiment, when N, P, K, and $CaCO_3$ (no magnesium added) were added to the soil as in the original tchernoziem with the same fertilization. Experiments without potassium were not carried out again.

Plants are thus able to utilize the elements magnesium and potassium in a soil from which the exchangeable bases of these elements were removed practically completely. When the other nutritive elements were provided, high yields were obtained (10 gm. of oat grain per pot corresponds to about 4 tons per hectar).

Totally different results are obtained concerning the rôle of calcium in plant nutrition: if practically all the exchangeable calcium is removed from the soil and is then replaced by a base, the presence of which does not prevent the development of the plant in one way or another, then, as the experiments here reported show, the plant dies entirely, when no calcium salts are added to the soil.

Thus, for example, the crop of oats in the original tchernoziem with complete fertilizer (N, P, K) was 6 gm. per pot (small pots with 600 gm. of soil). In the soil with complete fertilizer and fully saturated with magnesium, the plants died completely. A yield of 2.5 gm. was obtained, however, when $CaCO_3$ and complete fertilizer were added. The same picture was obtained, as will be seen later, when the soil was saturated with other bases as well.

It is quite clear that in our methods of replacing the exchangeable bases from the soil, the plant in a soil practically freed from exchangeable magnesium and potassium finds in the same soil a source of these elements which are essential for a certain yield; however, in a soil freed from exchangeable calcium, the plant does not find another source of this element in the soil itself.

It is easiest to explain this phenomenon by supposing that by our methods of replacing exchangeable bases we do not replace them completely, in spite of very prolonged washing of the soil with a solution of a salt of the given replacing base. This is actually so, since even after 20 repeated treatments of a soil with a solution of the replacing cation, we cannot remove completely from the soil all the extensively exchangeable bases, whereby, as we have seen, there is reason to suppose that, after such treatment, there are left, of the exchangeable bases in the soil, more magnesium and potassium than calcium; there is left enough of the first two in the soil to allow a normal development of the plants, provided the other nutritive elements are present, to give certain crop yields, while there is left so little calcium as to prevent plant development.

We will now take up the major subject of this paper. Among the numerous questions concerning the exchangeable bases in soil and their relation to plants, the first to be studied was, in my opinion, the reaction to plants of a soil fully saturated with one base or another, either those found naturally in the soil or not. These experiments were begun in 1913; one experiment was carried out

using a tchernoziem saturated with either NH_4, Na, or K.   As a result of the complexity of preparation of a soil saturated with these bases and at the same time freed entirely of the salts used for treating the soil to bring about such a saturated condition, and also as a result of the certainty that agricultural plants will not develop in such soils, this experiment was carried out in tumblers containing 200 gm. of soil.   Complete fertilizer (N, P, K, and Mg), without and with $CaCO_3$, was added to the soil.   The result was that the seeds of all agricultural plants, both in the presence and in the absence of $CaCO_3$, failed to germinate, rotted, and perished.   The soil saturated with NH_4, Na, or K was thus found to be a medium totally unsuited for the development of agricultural plants.   The causes of this unsuitability are at present, of course, well known to us.

We planned to study further the development of plants in a series of soils having different exchange capacities and saturated to a different degree with these various bases; in respect to the exchangeable NH_4, to study the need of soil in nitrogen fertilization, using such quantities of exchangeable ammonia in soil as are not injurious to plant growth.   Such investigations were not only of theoretical but also of practical interest, especially as to our knowledge of alkali soils.   This problem could not be carried out, however, and the continuation of our experiments concerning the conditions of growth of plants in soils saturated with one base or another could be undertaken only in 1928.

Soils saturated with the bases Mg, Ba, Mn, Co, Ni, Cu, and H were prepared from a tchernoziem rich in humus.   The experiment was carried out in small vessels using 700 gm. of soil.   In view of the small quantity of the various soils thus prepared only one plant was employed in this experiment, namely mustard.   The soils received either no fertilizer or a complete fertilizer (0.1 gm. N as $NaNO_3$, 0.1 gm. P_2O_5 as NaH_2PO_4, and 0.05 gm. K_2O as K_2SO_4), but no $CaCO_3$.   The study of the influence of $CaCO_3$ upon the same soils was postponed to the following year.

The plants yielded a crop (quite a normal one) only in the original tchernoziem.   In the case of the soils saturated with each of the foregoing bases, however, the plants either did not germinate or appeared in the form of a few seedlings which soon perished, in spite of repeated planting.

The soils taken from the experiment of 1928 were placed in 1929 in 600-gm. quantities, in pots, and complete fertilizer (in the same quantities as in 1928) was added to all pots containing the original soil.   The pots of the remaining soils that were without fertilizer in 1928, received complete fertilizer.   The pots that received complete fertilizer in 1928 did not receive any new portion of it since it was not used at all in 1928, but 10-gm. quantities of $CaCO_3$ were added.   Oats were the plants used in this experiment.

The following experimental results were obtained (per pot, on the average of two duplicate pots):

Original soil:
    Complete fertilizer — total yield 5.7 gm., grain 1.6 gm.
    Complete fertilizer + $CaCO_3$ — total yield 5.4 gm., grain 1.7 gm.

The soils saturated with various bases and treated with complete fertilizer but without any $CaCO_3$ gave the same results as in the previous year: either the seed did not germinate at all or the seedlings soon perished.

In the presence of complete fertilizer and $CaCO_3$, the results of last year were repeated in the soils saturated with Ba, Ni, and Co; the plants did not develop at all in these soils and the crop yield was 0; thus, when the three bases completely saturated the full capacity of absorption of a given soil, the oats could not develop, even when the soil was supplied with all nutrients (including calcium).

The soil saturated with Cu, supplied with a complete fertilizer and $CaCO_3$, gave a crop, but of an inconsiderable amount—0.39 gm. of total plant material and 0.05 gm. of grain (5 seeds per pot).

The soil saturated with Mg, supplied with a complete fertilizer and $CaCO_3$, gave a total crop yield of 1.56 gm. and 0.38 gm. of grain.

TABLE 2

Yield of oats on a tchernoziem rich in humus and saturated with various cations

SOIL SATURATED WITH	COMPLETE FERTILIZER		COMPLETE FERTILIZER + CaCO₃	
	Total crop yield	Grain	Total crop yield	Grain
	gm	*gm*	*gm*	*gm*
Original soil	5 9	1 44	5 6	1 20
Mg	0 33	0 01	2 4	0 62
Ca	6 3	1 92		
Sr	5 4	0 8	5 6	0 97
Cd .	0	0	0	0
Fe⁺⁺	0 7	0 14	1 6	0 35
Fe⁺⁺⁺	0	0	1 0	0 23
Al	0 24	0	2 6	0 80
H	0 21	0 1	5 7	1 6

The soil saturated with Mn supplied with a complete fertilizer and $CaCO_3$, yielded 1.6 gm of total crop and 0.18 gm. of grain.

Finally, the soil saturated with hydrogen, supplied with a complete fertilizer and $CaCO_3$, gave a crop yield of oats equivalent to that of the original soil.

These experiments fully confirmed the results obtained in 1912: if practically all the exchangeable calcium is replaced by any other base (in this case by H, Mn, and Mg), the plants will develop only when a calcium salt is introduced into the soil, but if practically all the magnesium in the soil is replaced by another base (in this case by H and Mn), the plant, if it is at all capable of growing in the presence of this saturating base, will develop and give a normal crop yield even without the addition of a magnesium fertilizer.

During 1929, vegetation experiments were carried out in soils saturated with a number of other bases, namely Mg, Ca, Sr, Cd, ferrous and ferric iron, Al, and hydrogen. The experiments were carried out in the same vessels as

in the previous case, with complete fertilizer (N, P, K, Mg), as well as with complete fertilizer and $CaCO_3$. Oats were used as the crop. The yields thus obtained are given (the average of two parallel pots) in table 2.

In this experiment as well, the soils saturated with most of the bases studied, in the absence of $CaCO_3$ gave either no plant growth at all (Cd and Fe^{+++}) or gave very limited crop yields (Mg, Al, H). A higher crop yield was obtained, in the absence of $CaCO_3$, in the soil saturated with ferrous iron (it is possible that the exchangeable calcium was not completely removed from the soil). A high crop yield was obtained, in the absence of $CaCO_3$, in the soil saturated with calcium (about the same as in the original soil). The reason for this is quite clear. But quite unexpectedly the crop yield on the soil saturated with Sr, in the absence of $CaCO_3$, was about the same as in the original soil or as in the soil saturated with Ca (considerably less grain). If we consider the data for all the other exchangeable bases here studied, we must recognize that in the soil saturated with strontium, the plants can do without available calcium, a phenomenon not taking place in the case of the other cations tested; the conclusion suggests itself that strontium may to a certain extent take the place of calcium for plant growth.

The introduction into the soil used in this experiment of $CaCO_3$ in addition to N, P, K, and Mg has markedly changed the general picture. Of the eight soils saturated with various bases (namely Mg, Ca, Sr, Cd, Fe^{++}, Fe^{+++}, Al, and H) a crop yield was obtained only in the case of calcium and strontium and possibly also of ferrous iron. In the same soils treated with $CaCO_3$ only the soil saturated with cadmium gave no crop; cadmium saturated the base exchange of the soil and killed the oats entirely. The plants grew on all the other soils, giving a crop of varying magnitude. A crop of about the same magnitude as on the original soil was given by the soil saturated with hydrogen (as in the previous experiment) and by the soil saturated with strontium. In the case of all the other exchangeable bases, the plants grew but gave a considerably lower yield: in the case of the soil saturated with Mg or Al, the crop was about half the size of that in the original soil, while the ferrous and ferric iron saturated soils gave even lower yields.

We may consider it definitely established at the present time that various physical and chemical soil properties, including not only those of the solid phase but also of the liquid and gaseous phases, are closely related to the composition of the bases saturating the exchange capacity of the soil. We now know that of the various elements entering, in appreciable quantities, into the composition of the soil (Na, K, Mg, Ca, Al, Fe, H, as well as Mn, NH_4, and Li), calcium occupies, as an exchangeable base, a special rôle in the life of the soil. First, it is usually present in soils (at least in those which are not too much destroyed) in considerably larger quantities than all the afore-enumerated metals, in the form of exchangeable bases. Secondly, when calcium saturates or almost saturates the exchange capacity of the soil, there are created in the soil conditions most favorable for supporting in the soil solution a reaction close to

neutrality; the elements Li, NH₄, Na, K, and partly Mg, as exchange bases, bring about a more alkaline reaction, whereas Mn, Al, Fe, and H bring about an acid reaction. Third, the exchange calcium creates in the soil special physical conditions, as a result of which a mutual relation is established between the aqueous and gaseous portions of the soil which is most favorable, in a given soil and given climatic conditions, for the growth of most higher plants and aerobic microörganisms. In the case of all the cations, as exchangeable bases, afore-enumerated, this relation is less favorable. Magnesium is nearest to calcium in all these respects.

As a result of the afore-reported vegetation experiments 'in soils fully saturated with one of the following 16 bases: H, NH₄, Na, K, Mg, Ca, Sr, Cd, Ba, Mn, Fe⁺⁺, Co, Ni, Cu, Al, Fe⁺⁺⁺, we find that the plant as well points to the very special position of exchangeable calcium among all the bases tested.

We have seen that, on the one hand, in a soil not containing exchangeable calcium, the plant does not develop at all, if some calcium salt is not added to the soil to serve as a source of calcium for the plant; that portion of calcium of the soil which is not exchangeable (at least intensively), as opposed to the unexchangeable magnesium and potassium, cannot be assimilated by plants in sufficient quantities required for their growth; on the other hand, the experiments have shown that this base may fully saturate the exchange capacity of the soil and the plant will develop in such soil, as measured by crop yield, as well as in a natural soil which contains, in addition to exchangeable calcium, exchangeable magnesium.

Of all the 16 cations tested in saturating fully the exchange capacity of the soil, only one other, namely strontium, which is very close to calcium in its properties, proved to be closely related to calcium also in its action on plants. In the soil saturated with this base, i.e. not containing any exchangeable calcium, the oats develop almost as well as in the soil containing exchangeable calcium. The total yield of oats in this soil was almost the same as that of the original tchernoziem and that of the tchernoziem saturated with calcium, but the grain yield was less. Thus, strontium-saturating a soil completely, on the one hand, failed to injure the plant, just as when the soil was saturated with calcium, and, on the other hand, strontium seemed to have replaced calcium as a nutritive element for plant growth.

All the remaining elements saturating completely the exchange capacity of the soil and thus removing the available calcium necessary for plant nutrition produced soils on which the plant, if it developed at all, grew only when CaCO₃ was added to the soil. Of these elements, let us consider first the hydrogen ion.

The experiment shows that in a soil fully saturated with H, the oats did not develop at all, in the absence of CaCO₃. Two causes prevented their growth: the absence in such a soil of available calcium and the acid reaction. The introduction of CaCO₃ removed these causes, and the soil gave then as large a yield as the original tchernoziem or the tchernoziem saturated entirely with calcium. The introduction into such soil of CaSO₄ in place of CaCO₃ showed

that the growth of oats was prevented not only by the absence of sufficient calcium available for plant growth, but also by the unsaturated condition of the soil with bases (to be more exact, those free acids which were formed in such soil as a result of exchange of the bases of the salts in the soil solution by the hydrogen ion of the unsaturated soil).   The results were as follows:

(Soil fully saturated with hydrogen)

Complete fertilizer	Total crop yield	Grain
	gm	gm
$-CaCO_3$	0 21	0 10
$+CaSO_4$	0 24	0 02
$+CaCO_3$	5 70	1 60

Of the remaining bases studied, all, i e. NH_4, Na, K, Mg, Cd, Ba, Mn, Ni, Co, Cu, Fe^{++}, Fe^{+++}, and Al, when they saturated completely the exchange capacity of the soil, in the absence of $CaCO_3$, brought about the complete death of the oats   In the presence of $CaCO_3$ in soil saturated with some of these bases, namely, NH_4, Na, K, Cd, Ba, Ni, Co, Cu, the oats also perished; the soil saturated with these bases gained chemical properties[2] which prevented plant development even in the presence of all the elements necessary for growth   It seems that one deals here with those toxic products which are produced in the soil solution of these soils   These bases, which enter the soil solution, as a result of their replacement from the soil by the cations of the nutrient salts added or by the hydrogen ion of water form such products.   It is evident therefore that in the case of the exchangeable NH_4, Na, and K, the toxicity is brought about not by these bases themselves but by the free hydroxyl ions liberated in their presence   The remaining bases (Cd, Ba, Ni, Co, and Cu) seem to destroy the plant by being directly toxic, and not as a result of the formation in the soil solution of a reaction unfavorable for plant growth, the reaction can be changed only to a limited extent by the introduction of these bases into the soil, and even then it will be toward acidity, which, under the conditions of our experiment (the presence of $CaCO_3$), cannot have an injurious effect upon plant growth

Finally, in the soils saturated with one of the following bases: Mg, Mn, Fe^{++}, Fe^{+++}, Al, the oats perished in the absence of $CaCO_3$, but grew in the presence of this compound, giving, however a lower crop yield   Although the original tchernoziem, saturated with calcium alone, gave about 6 gm. of the crop per pot, the soils saturated with these bases + $CaCO_3$ gave the following yields:

	gm
Mg .	2 4
Al .	2 6
Mn	1 6
Fe^{++}	1 6
Fe^{+++}	1 0

[2] Under the conditions of our experiments with the soils saturated with bases, the special physical properties of these soils could not play any important part in the death of the plants.

These bases, when fully saturating the exchange capacity of the soil, are thus found to be also toxic to plants, although to a less extent than reported previously. Of these bases, magnesium and aluminum are less injurious than manganese or the oxides of iron.

The foregoing considerations point to the special rôle of exchangeable calcium in the life of plants. *Of all the cations investigated, as exchangeable bases in soil, only calcium (and evidently to the same extent, strontium) creates in the soil, when fully saturating its exchange capacity, conditions most favorable for plant life.*

The question concerning the influence of various combinations in the composition of exchangeable bases in soil upon plant growth was not touched upon at all in the aforementioned experiments. The fact, for example, that barium, when fully saturating the base exchange capacity of the soil, destroys the vegetation entirely, cannot as yet lead us to conclusions concerning the influence of this base upon plants when it saturates in the soil only a part of its base exchange capacity while the remaining part is saturated by calcium. It is quite possible to admit, if not in respect to barium, at least in respect to some of the other toxic bases, that when present in very small amounts in the soil absorbing complex, they will not only be uninjurious to plant growth, but may prove to be stimulants, as found in the case of salts of certain elements. The great interest and the possibly considerable practical importance of the further continuation of these experiments in greater detail thus become evident.

An attempt was made in this connection to study the influence of various relations between the exchangeable calcium and magnesium in soil upon plant growth. Sixteen samples of soil were prepared from the tchernoziem with varying ratios óf exchangeable calcium and magnesium. These soils were used in 1929 for a vegetation experiment with oats carried out at the Nossov Agricultural Experiment Station. Unfortunately, because of unfavorable conditions, the checks in the duplicate pots were so inadequate that it was impossible to draw any conclusions from the crop yields in the small pots. However, the development of the oats in this experiment has shown clearly that within the limits of minimum content of absorbed magnesium in the original tchernoziem and the maximum in the soil saturated with magnesium, there were pots with an optimum plant development. It is evident that a certain increase of the quantity of exchangeable magnesium at the expense of exchangeable calcium has exerted a favorable effect upon the oats in the tchernoziem under investigation. This question concerning the exchangeable magnesium is doubtless of a very practical interest, since it is connected, for example, with the question of the amount of magnesium permissible in the lime used for liming of soil. It is possible that the special care which some persons exert in this connection may prove to be unnecessary. It is interesting to note that, according to the data obtained in this experiment (data which are, however, not fully dependable), the oats were not affected injuriously when a large part of the exchangeable calcium of the soil was replaced by magnesium. These experiments will be repeated.

Let me call attention further to the fact that in respect to exchangeable sodium, which when fully saturating makes the soil, totally unsuitable for cultivation, theoretical considerations (2) definitely speak in favor of the presence of a small quantity of it in a tchernoziem, creating at least favorable conditions for nitrogen nutrition. This was confirmed by a small experiment carried out a few years ago at the Nossov Agricultural Experiment Station. This experiment will be repeated on a larger scale.

CONCLUSIONS

The growth of oats and, in some cases, of mustard and buckwheat in a tchernoziem rich in humus was studied; the exchange capacity of this soil was saturated with one of the following bases: H, NH_4, Na, K, Mg, Ca, Sr, Cd, Ba, Mn, Fe^{++}, Co, Ni, Cu, Al, Fe^{+++}.

The crops gave the same yield as in the original tchernoziem only in the soil saturated with calcium. The soil saturated with strontium gave almost as large a yield as the original soil or the soil saturated with calcium; this soil needed no calcium fertilization

The plants did not grow at all in the soils saturated with each of the remaining bases, both without fertilization and with nitrogen and phosphorus fertilization. When, however, $CaCO_3$ was added to the soil, in addition to nitrogen and phosphorus, a normal crop, similar to that in the original soil, was obtained only in the soil saturated with H; in the soils saturated with Mg, Mn, Fe^{++}, Al, Fe^{+++}, a crop was obtained, to a smaller extent, however, than in the original tchernoziem. The plants perished entirely in the soils saturated with all the other bases (NH_4, Na, K, Cd, Ba, Co, Ni, Cu), both in the presence and absence of $CaCO_3$.

After the practically complete replacement from the soil of exchangeable calcium, the plants require for their development the introduction into the soil of calcium fertilization, without which they do not grow at all, they are unable to utilize the unexchangeable calcium of the soil.

After the practically complete replacement from the soil of Mg and K, plants are able to develop and give a more or less normal yield even without the introduction into the soil of magnesium and potassium fertilizers; the plants are able to utilize the unexchangeable magnesium and potassium of the soil.

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AN IMPROVED SOIL SAMPLING TUBE

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During the past few years certain investigations in progress here at the Oklahoma Agricultural Experiment Station have required that a considerable number of soil moisture samples be taken. A soil auger has been used for most of these studies but there are many objections to a soil auger, some of which are as follows:

> Contamination of the deeper samples with soil from the wall of hole due to friction which occurs as the auger is withdrawn, especially when soils are wet.
> Slow penetration in case of dry or very compact soils
> Difficulty of removing soil from hole when it is dry or very sandy

Although the last objection can be overcome by using an auger with a sleeve as recommended by Tinsley and Vernon (9), the first two objections are not easily remedied.

King (3) has designed a soil sampling tube which has been used rather extensively by many investigators. Modifications of this tube are described by Kopp (4) and Veihmeyer (10). Other methods of obtaining soil samples have been used by Stevenson (7), Powell (6), and Green (2), and Neller (5), but they were not designed to obtain samples for soil moisture studies and are of use for taking only shallow samples.

The chief objections encountered in the use of soil sampling tubes are that they are very difficult to remove from many soils because of two factors, as follows:

> Friction between expanded portion of tube and soil face
> Suction developed in moist soils as tube is raised

In order to overcome these two difficulties several different tubes were designed and tested. Plate 1 shows a soil sampling tube on the left which is used by the staff of the Bureau of Chemistry and Soils, U. S. Department of Agriculture. In the center are two driving hammers and on the right are three sampling tubes designed to reduce the friction between the tube and the soil by making a series of tubes, the larger one for surface layers of soil and the smaller ones for the deeper horizons which are sampled. Veihmeyer (10) suggests decreasing the outside diameter of the cutting point on the tubes used to take the deeper samples; however, this does not overcome the problem of reducing the friction

which arises between the hole and the tube and which is often increased as a result of loose material falling in above the expanded portion at the lower end, and also it does not reduce the suction which develops when the tubes are used in moist soils.   Under favorable conditions the tubes shown on the
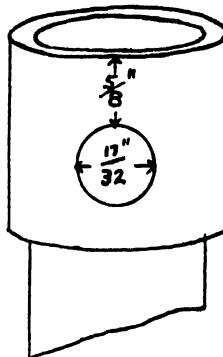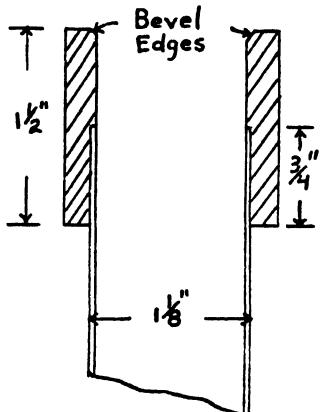
Fig. 1
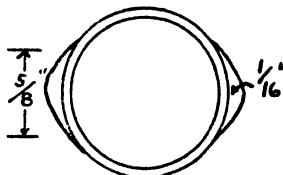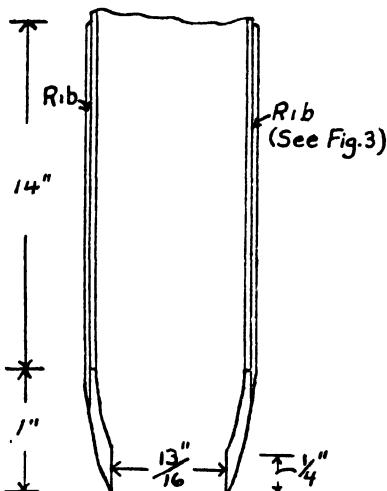
Fig. 2

Fig. 3

FIG 1. LONGITUDINAL SECTION OF SOIL SAMPLING TUBE MADE FROM 14-GAUGE SEAMLESS STEEL TUBING
FIG. 2. COLLAR OF SOIL SAMPLING TUBE SHOWING HOLE IN WHICH STEEL ROD IS INSERTED
FIG. 3. CROSS SECTION OF RIBBED PORTION OF SOIL SAMPLING TUBE

left of plate 1 can be pulled from the soil without a jack (8) and consequently this reduces the amount of time required to remove the sample.   However the use of a jack under any condition is objectionable and any procedure which will reduce its use to a minimum would be desirable.

Further studies on the design of a tube which would overcome the problem of friction between portions of the tube and the soil wall and also reduce the suction force encountered when the tube is driven into moist or wet soil resulted in the use of a rib attached to the side of the tube which increases the size of the hole when the tube is given a complete turn after it is driven to the desired depth. Also the tendency to produce a partial vacuum at the bottom of the hole is reduced because the tube does not have an expanded portion at the lower end, and when the ribs are moved, air can pass immediately into the bottom of the hole. Later investigations indicated that two ribs placed on opposite sides of the tube were better than one rib placed on the tube. A steel rod inserted through a hole in the collar of the tube has many advantages over handles welded to the collar of the tube. A design of the tube as recommended is shown in figures 1, 2, and 3. A suitable driving hammer can be made from a piece of steel shafting about 3 inches in diameter and 8 inches long. A hole should be bored in the center of each end of the shaft and steel rods about five-eighths of an inch in diameter and 18 inches long can be threaded and screwed into the holes.

For surface sampling in ordinary soils a 3-foot zone can be sampled with one tube of proper length. In very compact or dry soils only two 1-foot samples can be removed conveniently with each tube. For sampling soils to a depth of 6 feet, three tubes are recommended as follows:

Tube No. 1, $4\frac{1}{2}$ feet long, $1\frac{1}{8}$ inch in diameter
Tube No. 2, $5\frac{1}{2}$ feet long, 1 inch in diameter
Tube No. 3, $7\frac{1}{2}$ feet long, $\frac{7}{8}$ inch in diameter

Tube no. 2 is used only when very unfavorable conditions are encountered.

The tubes should be constructed of 14-gauge cold rolled seamless steel tubing and the collar and cutting point should be case hardened so that they will not batter from continued use. In case longer tubes are made, heavier gauge tubing should be used. The collar should be brazed to the tube but the steel ribs should be welded because steel takes a better polish than the copper alloy and consequently it does not adhere to the soil as readily as the brazed surface and the tubes can be turned in the hole and extracted from the soil with less energy than when the ribs are attached with brass.

Oiling the tubes will help materially in reducing the amount of energy required to pull them from the soil, when sticky soil is encountered.

In case loose soil falls into the hole it can be discarded when the tube is inverted and only the solid core saved for the sample.

When tubes of different diameters are used, the cutting edge should be about one-tenth of an inch smaller than the inside diameter of the tube. The cutting edge should be turned from a piece of steel shafting and welded to one end of the tube. In case tubes of larger diameter are constructed it may be necessary to increase the thickness of the ribs.

A steel rod one-half inch in diameter and 24 inches long is used to turn the

tubes after they are driven into the soil and serves as a handle by which they can be pulled.

For deep sampling an auger probably can be used to better advantage than tubes. Alway, McDole, and Trumbull (1) have recommended the equipment needed for both shallow and deep samples; however in tests made at this station a 4-inch post auger is preferred to a small auger for digging deep holes. Many holes have been dug to a depth of 22 feet at this station and less than one hour has been required to take samples from individual holes even when very compact, sticky soils are encountered.

In case of stony soils the large auger is also preferable because samples can often be obtained with it when they cannot be taken with either the soil tube or the small auger.

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PLATE 1

SOIL SAMPLING TUBES AND HAMMERS USED IN THIS INVESTIGATION
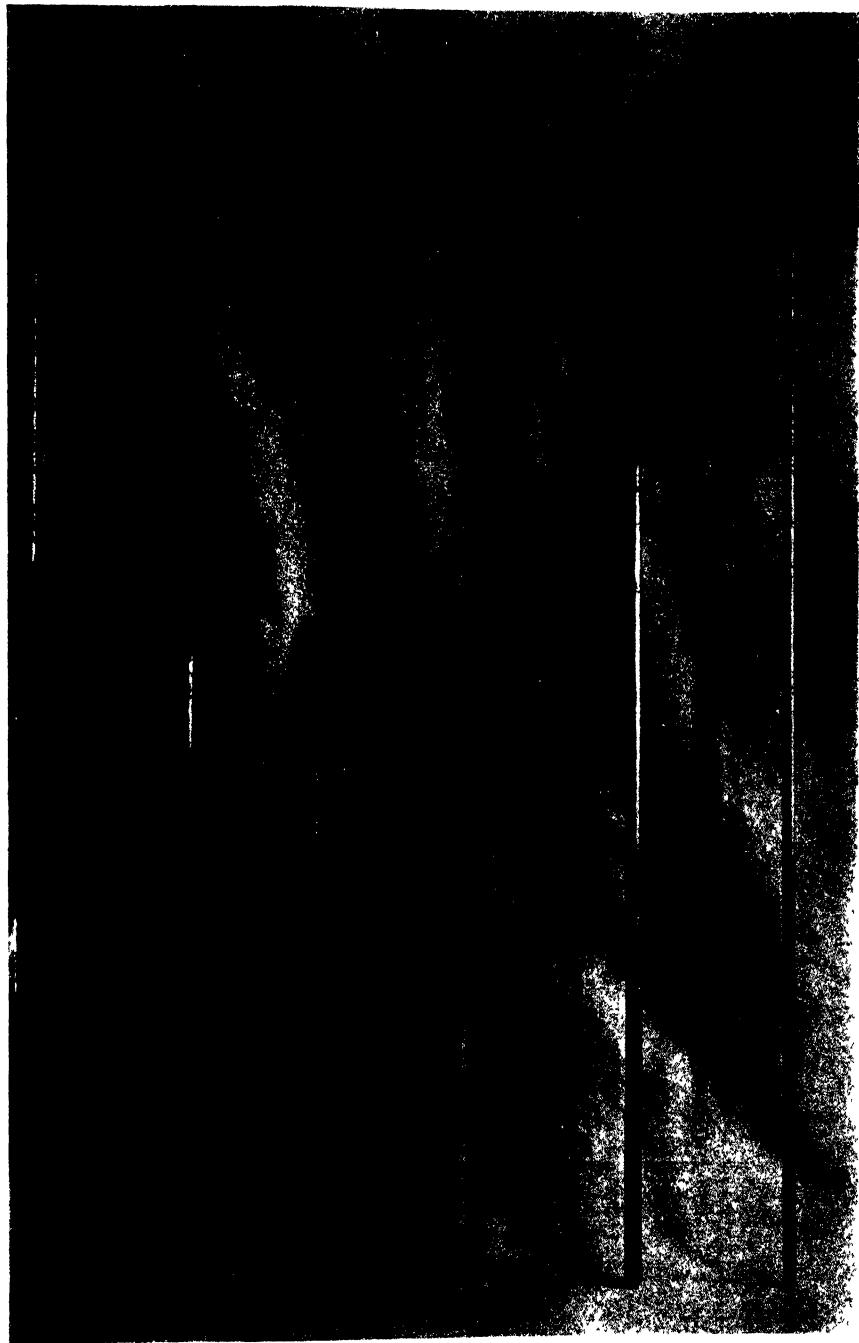
BOOK REVIEW

The Physical Properties of the Soil. By B. A. KEEN. Longmans, Green and Company, Ltd., London, 1931. Pp. vi + 380, figs. 93.

Dr. Keen is to be congratulated for the timely appearance of his book on the physical properties of the soil. He has exhibited the rare quality of a thorough scholar and the ability of a trained physicist in the preparation of a text that is a delight to read and a source of inspiration to serious investigators and students in this important field of applied physics.

The book contains much that has been produced by Rothamsted workers in recent years and a coherent and comprehensive survey of an extensive literature on a subject involving great complexity. Students who have a mathematical training will succeed best in the interpretation of certain parts of the text, although much of it is very thoroughly intelligible to students less fortunate. In fact, many farmers would enjoy and profit by reading such chapters as the historical introduction, the chapter on cultivation implements, and numerous paragraphs throughout the text. The discovery, which seems to be due to Dr. Keen and his associates, of a more or less permanent pattern of resistance to the plow on a visually uniform field is a challenge to the admiration and an explanation of a heretofore unexplained variability in random samples. One can scarcely fail to marvel not only at the significance of the isodyne record but at the mechanical device itself which rides modestly along on the plow and leaves a record of one of the most significant of all physical properties—the friability of the soil.

Certain phases of the science, such as the theory of mechanical analysis, the laws of movement of moisture, the elastic and plastic properties and processes, and thermal adjustments, seem to be crystallizing into permanent form. Colloidal chemistry and colloidal physics, such as they are, and the recent work with the exchangeable bases, have also been given careful consideration and the discussion will prove profitable and interesting. We shall hope with Dr. Keen, however, that the future will bring new enlightenment in these somewhat elusive aspects of soil physics.

The author has made a successful effort to give due consideration and recognition to contributions from various sources, and American investigators come in for much credit for the part they have played.

The book will serve a useful purpose as a text for agricultural students and will stimulate prospective young investigators to prepare themselves to wrestle with mathematics and physics as a schoolmaster for service in this basic industrial activity. This book will not be overlooked in America.

WILLARD GARDNER.

ANNOUNCEMENT

The price of Proceedings of the First International Congress of Soil Science will be increased on July 1, 1931, to $5.50 a set to members of the society in the United States and to $6.50 a set to members in foreign countries, according to a recent announcement of the Executive Committee of the American Organizing Committee of the Congress. This additional charge is necessary to take care of transportation cost.

ON THE DECOMPOSITION OF HEMICELLULOSES BY MICROÖRGANISMS: I. NATURE, OCCURRENCE, PREPARATION, AND DECOMPOSITION OF HEMICELLULOSES[1]

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In the decomposition of complex organic matter by microörganisms, many phenomena still remain obscure, largely because of an insufficient understanding of some of the chemical constituents of this organic matter and a lack of knowledge concerning the microörganisms bringing about the processes of decomposition. Only very recently has a simultaneous study been undertaken of the transformation of the various chemical constituents of plant residues in their decomposition by pure and mixed cultures of microörganisms (8, 30a, 90, 103). The results thus obtained have contributed to a better understanding of the decomposition processes that take place in manure and in soil, as well as to our knowledge of the origin of humus.

Of the various organic constituents in plant residues, attention has been in the past largely centered upon the decomposition of the sugars, celluloses, and proteins. Recently, the rôle of lignin in the degradation of organic matter under aerobic and anaerobic conditions has also attracted considerable attention, although very little is yet known concerning the microörganisms capable of bringing about the decomposition of this more or less resistant complex, and the mechanism of their action. Of the other major plant constituents, the hemicelluloses, a group of compounds second only to cellulose, as regards their relative abundance in plant tissues, have been studied only to a very limited extent, from the point of view of their decomposition by microörganisms. This was partly because of a lack of a clearer conception of the chemical nature of these compounds and partly because of an insufficient knowledge of the organisms concerned in their decomposition.

NOMENCLATURE AND COMPOSITION OF HEMICELLULOSES

The carbohydrates of plants are frequently classified into five groups: 1. Substances soluble in cold water; to this class being the mono-, di-, and tri-saccharides, as well as certain water-soluble polysaccharides. 2. Carbohydrates insoluble in cold water, but yielding sugar under the action of diastase and similar enzymes; here belong starch, glycogen, dextrins, and inulin. 3.

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[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

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Carbohydrates insoluble in water and resistant to the action of the enzyme diastase, but soluble in alkalies and readily hydrolyzed by hot dilute mineral acids to give reducing sugars; this group comprises the heterogeneous hemicelluloses; the pectins and gums are related to this group, although they give on hydrolysis reducing sugars, in addition to other complexes.   4. Cellulose, sometimes referred to as true cellulose, which is hydrolyzed by concentrated mineral acids giving glucose only.   5. Lignins, a group of compounds, cyclic in nature; these complexes are not polysaccharides at all, but have been frequently classified with them because of the fact that they are associated together in the cell membrane of plants.

The term "hemicellulose" was first introduced by Schulze (98, 99).   It was used to designate a group of substances entering into the composition of the cell membrane, similar to cellulose, but soluble in dilute alkali solutions and fairly readily hydrolyzed with dilute mineral acids, and yielding pentoses and hexoses.   The function of hemicelluloses in plants is both of structural carbohydrates, similar to that of cellulose, and of reserve carbohydrates, similar to that of starch.   The reserve hemicelluloses are found in kernels of seeds, especially those that are poor in starches and fats, the hexoses predominating as the products of hydrolysis.   The structural hemicelluloses are found in seeds, fruit, husks, wood, straw, etc., and give, on hydrolysis, largely pentoses.

A number of various ill-defined compounds including the gums, certain slimy plant products, and various sugar condensation products are considered to be allied to hemicelluloses.   The term "hemicellulose" has become so very confused and is so much misused, that it has recently been suggested (37, 41) that it be dropped altogether from scientific literature.   Hess (37) preferred to designate this group of compounds as "cellulose associates," whereas Wise (129) suggested that they be referred to as "the non-cellulosic polysaccharides of the cell wall."

Recently the tendency has been (66, 70, 73, 74) to consider the hemicelluloses not as true polysaccharides, such as pentosans and hexosans, but as compounds containing considerable quantities of acid groups (glucuronic, galacturonic, mannuronic, etc., termed collectively "uronic acids"); the pectic substances may be considered as typical examples of such complexes.   When pentosans are determined in plant materials by the "furfuraldehyde method" of Kröber (54), an error is frequently made in assuming that only the pentose units yield furfuraldehyde; the latter is obtained not only from the pentoses but also from the uronic acids.

$$C_6H_{10}O_7 = C_5H_{10}O_5 + CO_2$$
$$\text{uronic acid}\quad\text{pentose}$$

$$C_5H_{10}O_5 = C_5H_4O_2 + 3\ H_2O$$
$$\text{pentose}\quad\text{furfural}$$
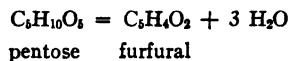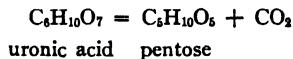
Hemicelluloses, gums, and pectins are thus found to consist of pentoses, hexoses, and uronic acids, in varying proportions.   Hemicelluloses from different

sources vary considerably in the products of hydrolysis, both qualitatively and quantitatively. O'Dwyer (74) found that beechwood contains two hemicelluloses, one of which yields on hydrolysis arabinose and galacturonic acid, and the other yields xylose and glucuronic acid. Norris and Preece (72) found four chemically and physically distinct hemicelluloses in wheat bran; two of these were true polysaccharides and two contained uronic acids. These and other (15, 65) investigations led Candlin and Schryver (13) to suggest the term "polyuronides" for complexes in which uronic acids are found in conjugation with sugars, largely pentoses. This term was supposed to include pectins and structural hemicelluloses which are very similar, while the term "hemicellulose" was reserved for substances which yield only sugars and no uronic acids on hydrolysis.

O'Dwyer (75) suggested a division of the hemicelluloses into A and B groups, on the basis of precipitation with glacial acetic acid and alcohol from the akaline extract; the nature of the hemicellulose was found to depend on the age of the plant and its nature. Norman (70) found that oat straw contained 22.8 per cent hemicellulose; the hemicellulose A consisted of 11 per cent uronic acid anhydride, 79 per cent arabinose and xylose calculated as anhydro-arabinose, and 10 per cent anhydro-galactose; whereas the hemicellulose B contained 32 per cent uronic acid anhydride and 68 per cent arabinose. Rye straw contained 33 per cent total hemicellulose, the A fraction comprising 5 per cent uronic acid anhydride, 60 per cent anhydro-pentose, and 35 per cent anhydro-hexose, whereas the B fraction was made up of about 29, 60, and 11 per cent of the respective three complexes. No fundamental difference was found (71a) to exist between the gums and the hemicelluloses.

For the present, before a more exact characterization and classification of these carbohydrates have been decided upon, we may refer to "hemicelluloses" as those polysaccharides which are insoluble in water or soluble with difficulty, but which are soluble in dilute alkalies and acids; they are readily hydrolyzed by hot dilute acids at ordinary pressure, giving on hydrolysis either pure monosaccharides (hexoses and pentoses), mixtures of monosaccharides, or a mixture of monosaccharides and uronic acids. These polysaccharides are not acted upon by diastase, inulase, or related enzymes, but some of them may be acted upon by certain special enzymes, such as cytases. The limits of this definition are evidently not very sharp. The hemicelluloses will thus comprise a wide variety of compounds, with pure polysaccharides related to starch and inulin at the one extreme and pure uronic acid complexes at the other; an illustration of the latter is found in algin or alginic acid, isolated from marine algae, which represents a polyuronic acid, in which all the carboxyl groups are free and all aldehyde groups conjugated (67). Most of the hemicelluloses will be found to contain both an acidic nucleus and a sugar fraction. The gums, mucilages, and pectins will thus be classified with the hemicelluloses; some of these substances, especially the pectins, may contain also other chemical groups, such as methyl alcohol and acetic acid.

OCCURRENCE OF HEMICELLULOSES IN NATURE

Hemicelluloses are widely distributed in the entire plant kingdom. They also occur abundantly in the bodies of microörganisms and in soil, having been introduced there by the plant residues or having been synthesized by the soil microbes. Hemicelluloses are particularly abundant in cell walls (58), in plant membranes (94), in root-stocks, rhizomes, and tubers (104), in wood (113), in cereal crops (86), and in a number of various seeds (16). These seeds usually contain also the corresponding hemicellulose-splitting enzymes or cytases; a number of higher animals were found to produce similar enzymes (88). Schulze (100) obtained from wheat and rye bran a hemicellulose which yielded arabinose and xylose on hydrolysis. Schulze and Pfenniger (101) later reported that the pods of *Pisum sativum* and *Phaseolus vulgaris* are rich in hemicelluloses, the content increasing with maturity of pods; on hydrolysis, galactose and arabinose were obtained. Indian clearing nuts were found (2) to contain a hemicellulose which gave on hydrolysis mannose and galactose, whereas that of ivory nut gave levulose and mannose. The hemicelluloses of American white oak (73) consist of 70 per cent pentosan (largely xylan, some araban) and 30 per cent hexosan, giving on hydrolysis mannose and galactose. The hemicelluloses or "gums" produced by bacteria are usually of the nature of compounds of various hexoses and pentoses with uronic acids (35). The literature dealing with the occurrence of hemicelluloses is most extensive, therefore no attempt will be made to review it here. Attention will be called to only certain specific hemicelluloses and their occurrence among plants, animals, and microörganisms.

Table 1 shows the relative abundance of hemicelluloses in fresh plant materials, in decomposed residues and in soil organic matter, as calculated from the reducing sugar produced on hydrolysis of the sugar and starch-free material with hot dilute hydrochloric acid.

Of the various individual hemicelluloses, the pentosans have received most consideration. This is no doubt both because of the considerably greater abundance of these carbohydrates in nature and because the determination of pentosans by the furfuraldehyde method is less complicated than in the case of the hexosans. Pentosans have been reported to be present in all parts of the apple tree and its fruit (19), in seeds (28), in the cotyledons of seeds (63), to a great extent among the sea weeds (64), and in lower (20), as well as higher fungi (126); they have also been reported to be present in soil (18). Agricultural crops, such as rice (48), corn fodder (82), wheat (108), and various other plants (112) contain pentosans as an integral part of their tissues. Extra precaution is needed to free the various forms of wood cellulose (44) from hemicelluloses. The furfuraldehyde method does not differentiate between the various pentosans. Even various uronic acids, such as glucuronic and galacturonic, which occur widely in plant products, will be reported by this method as pentosans. This is true especially of composts, peat and soils,

when nearly all the "pentosans" may be due to uronic acid complexes, as will be reported in detail later.

The distribution of the individual pentosans is almost as wide as that of the hemicellulose group as a whole. A marked increase in the pentosan content of

TABLE 1

Quantitative distribution of hemicelluloses in various plants, plant products, and soils

KIND OF MATERIAL	PER CENT OF TOTAL HEMI-CELLULOSE	REFERENCE
Alfalfa tops	13 14	118
Rye straw	26 41	120
Young corn stalks	20 38	118
Mature corn stalks	21 91	118
Young pine needles	14 68	118
Old pine needles	18 98	118
Green oak leaves	12 50	118
Mature oak leaves	15 60	118
Carex (upper growing portion)	18 36	124
Carex (rhizomes)	20 86	124
Hypnum	18 92	124
Sphagnum (upper growing portion)	30 82	124
Sphagnum (lower dead portion)	24 50	124
Fallen needles of *Pinus strobus*	18 98	124
Chestnut wood (sound)	15 23	122
Cypress wood (sound)	11 16	122
Non-fibrous decomposed wood	4 72	122
Fibrous decomposed wood	14 43	122
Fossilized wood from peat	8 15	122
Fossilized oak wood	3 79	122
Composted rye straw	14 77	120
Composted horse manure	12 67	120
Horse manure (fresh)	23 52	121
Cow manure (fresh)	18 57	121
Sheep manure (fresh)	18 46	121
Lowmoor peat (surface)	10 31	124
Lake peat	12 14	124
German highmoor peat		
Younger sphagnum	16 88	124
Grenzhorizont	8 44	124
Older sphagnum	9 08	124
Woody peat	4 91	124
Pure wood from peat	5 06	124
Natural forest humus	15 28	125

the plant was found to take place with an increase in the maturity of the plant; the formation and accumulation of pentosan were thus observed to run parallel with those of the cellulose (117, 123). Schwalbe and Becker (102), however, reported that an increase in the lignin content of wood is accompanied by a

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decrease in the pentosan content, they suggested that pentosan is polymerized to lignin.   Similar observations were made by other investigators (24).   Table 2 shows the relative abundance of pentosans in various plants and plant prod-

TABLE 2

The occurrence of pentosans in plants, plant products, and soil

KIND OF MATERIAL	PENTOSAN	REFERENCE
	per cent	
Ripe soy-beans	3 4	6
Green soy beans	3 6	6
Corn fodder	21 8	82
Corn hulls	48 6	83
Corn endosperm	1 7	83
Corn germ	8 4	83
Barley grain	12 6	116
Barley husks	6 5	116
Barley embryo	5 2	116
Rye straw	22 9	118
Timothy hay	22 0	121
Young leaves	5 3	16
Old leaves	9 7	16
Fungi (six varieties)	0 86–1 17	20
Wood fungi	1 20–6 50	126
Soils	0 05–2 50	103
Forest soil	0 75	25
Garden soil	0 39	25
Poor sandy soil	0 04	25
Lowmoor peat (surface horizon)	4 7	124
Forest peat (surface horizon)	0 75	124
Highmoor peat (surface horizon)	7 52	124
Nettles (bast fibers)	14 0	91
Fir	11 48	84
Pine	10 80	84
Birch	25 86	84
Poplar	22 71	84
Beech	24 30	84
Ash	23 68	84
Willow	23 31	84
Alder	22 94	84
Wood fissils	0–0 4	84
Brown coal (lignite)	0 3–0 4	84
Hard coal	0	84

ucts, as calculated from the total yield of furfural, obtained on boiling with 12 per cent hydrochloric acid.

The wood of dicotyledenous plants is very high in pentosan, containing 17 to 27 per cent of this type of hemicellulose, whereas the wood of gymnosperms contains only about 6 to 12 per cent pentosan and a considerable amount of

hexosan-hemicelluloses (17). König and Becker (52) have shown, for example, that the pentosan content of spruce (*Picea excelsa*) and of pine (*Pinus silvestris*) was 11.30 and 11.02 per cent respectively, whereas the pentosan content of beech (*Fagus silvatica*), birch (*Betula verrucosa*), and poplar (*Populus tremula*) was 24.86, 27.07, and 24.75 per cent respectively. The mature stalks of cereals are almost as rich in pentosans as are the various species of wood. Soils and peats contain comparatively low amounts of pentosans, as compared with the materials that enter into their formation. It is also of interest to note that lower filamentous fungi contain about 1 per cent pentosan, whereas higher fungi, such as the mushroom and wood destroying fungi may contain as much as 8.0 per cent. Rege (85) reported, for example, that *Coprinus* sp. separated from decomposing straw contained 7.82 per cent pentosan.

Xylans, methyl pentosans, and arabans usually occur together and closely associated in the natural state. Xylan is found to the greatest extent in wood (111). In some species of wood, xylan is the only hemicellulose present and can be isolated in an almost pure form with comparative ease, especially by the method proposed by Schmidt (94). *Fagus silvatica* was shown (95) to contain two xylans, one readily soluble and one soluble with greater difficulty. Straw (36) also contains large amounts of xylan, whereas plants like *Cucurbita pepo* (14) contain xylan in smaller amounts. Combinations of xylan and arabans have been observed in wood (73) and in sugar cane (10). In the latter case about four parts of xylan are found to every part of araban. In *Allium cepa* (33) xylan is found associated with a methyl pentosan. Xylans are commonly associated with glucosans, and arabans with galactans (101, 111). The xylans frequently form such a firm compound with cellulose as to be removed only by the action of strong acids or alkalies (94). The hemicelluloses of pine wood were found to consist of an easily and of a difficultly hydrolizable portion. The first comprised 18 per cent of the wood, and gave on hydrolysis, 17 per cent pentose, 42.7 per cent mannose, 4.2 per cent galactose, 3.2 per cent galacturonic acid, 4 per cent fructose, and 28.9 per cent glucose (32).

A methyl pentosan is a substance yielding methyl-furfural by distillation with 12 per cent HCl, in contrast to the furfural produced from the pentosans. Methyl pentosans occur in cereals (110), in onions (33), in the seeds of leguminous plants (63), in sea weeds (89), and are distributed in small amounts in a variety of plants (6, 57). Rice was found (48) to contain, on the average, 1 per cent methyl pentosan, and corn fodder, approximately 0.4 per cent (82).

Next to the pentosans, the mannans have received the widest study. Their general occurrence has attracted more attention than their relative abundance. It is to be regretted that not only the mannans, but also all the other hexosan-hemicelluloses, have been studied so little with respect to their quantitative occurrence. Mannans are found in asparagus seeds (12), in wood (73), in ivory nut, and in salep (36). In pine wood (106), a large amount of mannan is found in late summer and in early autumn. Sulfite pulp (38) contains considerable amounts of mannan. Various Oriental plants (114), algae, and sea

weeds (47, 49, 64, 77, 80, 87) are rich in mannans. The "mannans" as well are frequently preparations which give more than one sugar on hydrolysis, as in the case of the gluco-mannan, found in the leaves and fibers of *Amorphophallus konjac*, which gives glucose, mannose, and fructose, in varying ratios, such as 1:2:0, 2:5:0, or 2:3:1 (69a).

The galactans are neither as widely distributed (54) nor found in as large quantities as the pentosans. It has been shown conclusively that they are very abundant in sea weeds (3, 29, 53, 64, 93, 77). A substance isolated from Chinese moss (*Sphaerococcus lichenoides*) and originally called "gelose" (69) was found to be a galactan. In other cases, galactans have been found in the germinating embryos of carob, in *Nux vomica*, in fenugrec and lucerne (7), in onion (33), in cell walls of wood (58), in white oak (73), in western larch (97), in legumes (101, 56), and in fungi (21). From the quantitative standpoint, very small amounts are found in sugar cane (9) and as much as 13 per cent in *Abelmoschus Manihot* (79). Galacto-mannans have been found in date stones (36). Galacto-arabans were found in legumes, gum tragacanth, cherry and peat gums, in arabinic acid, etc. The function of galactose and galacturonic acid in the pectin molecule has been discussed in detail by Ehrlich (24).

Dextrans are synthesized by microörganisms as well as by higher plants. They have been reported in yeasts (59), in bacteria (61), and in fungi (127, 128). Certain species of sea weeds (64) contain dextrans associated with the other groups of hemicelluloses. The dextrans of wheat, rye, and barley have been divided into α- and β-amylans (1, 78). The α-amylans are soluble in hot water whereas cold water is sufficient to bring the β-amylans into solution. In *Asparagus officinalis* (12) the dextrans are combined with the mannans. Considerable quantities of hexosan-hemicelluloses have been found in wood, especially in evergreens (84). Various species of wood contain the following percentages of hexosans: fir, 13.58; pine, 12.78; birch, 4.61; poplar, 2.60; beech, 4.36; ash, 5.70; willow, 5.05; and alder, 22.94.

METHODS OF ANALYSIS OF HEMICELLULOSES

From what has been said before, one can readily recognize that the chemical structure of the hemicellulose group is not always definitely established. What knowledge there is of their structure does not even indicate whether a galacto-mannan is a polysaccharide built up of the residues of galactose and mannose or whether it is simply a mixture of galactans and mannans (84). Numerous investigations have shown that the hemicelluloses are associated with other complexes within the plant. In fact, a pure hemicellulose has never been found by itself in nature. Usually it is combined with the other complexes, but frequently it may simply occur alongside of them.

The methods of analysis of hemicelluloses are still purely conventional (39), as is shown by the lack of uniformity of the results. The usual procedure consists in hydrolyzing the material with dilute mineral acids (2 to 3 per cent) at boiling temperatures. Lower results are obtained by heating under pressure

at 118° to 125°C. with 1 per cent acid than by heating with 3 or 5 per cent acid at the temperature of boiling water. Different concentrations of acids, heating at different temperatures, and different periods of heating all cause variations in the amounts of hemicellulose obtained. The difficulties arise because substances of indefinite characters are being extracted and hydrolyzed. As will be shown later, the various hemicelluloses exhibit great differences in solubility and in resistance towards hydrolysis. Further, there may be other substances of a non-hemicellulosic character present which will yield the same end products upon hydrolysis. It becomes evident, therefore, that a method employed for determining hemicelluloses in all plants must be purely conventional. If accurate determinations of hemicelluloses are required, the plant complex in question containing the hemicelluloses must be studied thoroughly to determine the concentration of the acid to be used, the temperature to be employed, and the length of time for hydrolysis to take place.

Upon being heated with water (105), some hemicelluloses pass into a state of colloidal sols which are extremely viscous and revert to non-rigid gels after standing a few days. Their solutions yield, when evaporated, continuous films which possess a considerable tensil strength and are much tougher than films of nitrocellulose. As a rule, they are not acidic in character. The viscosity of these substances is increased by alkalies and boric acid, and decreased by heat, mineral acids, iron and copper salts, hydrogen peroxide, sodium peroxide, and potassium persulfate. They form adsorption compounds with the hydrates of calcium and barium. Heavy, solid, white, opaque gel formation takes place when basic lead acetate, potassium permanganate, or Fehling's solution is added. These viscous gels have such a high surface tension that water cannot penetrate. It is the hemicellulose content of the sea weeds that causes them to gel; agar-agar is a typical representative of that group of colloids (62); the addition of electrolytes lowers the swelling capacity of agar-agar.

The idea (70, 74) that hemicelluloses are not true carbohydrates in the strict chemical sense, wherein the proportion of hydrogen to oxygen must be the same as that of water, has been discussed in the foregoing. The two types of hemicelluloses found in beech wood led to this conclusion; upon hydrolysis, one yields xylose and an amount of carbon dioxide corresponding to 11 per cent of glucuronic acid, and the other yields arabinose and an amount of carbon dioxide corresponding to 63 per cent galacturonic acid. Similar results have been obtained in the case of straw. This view has been partially confirmed (72, 94)

The pentosan group is more specific than the general hemicellulose group. Many observations have been made concerning the chemical features of the furfural-producing substances, or the pentosans. The range of solubility of the pentosans is not narrow. Upon the basis of solubility they have been divided (22) as follows: (a) those soluble in water; (b) those soluble in 1 per cent hydrochloric acid solution; and (c) those soluble in 12 per cent hydrochloric acid Of the pentosans in legumes (26), 44.50 per cent are dissolved

by 0.02 N acid and alkali solutions, 2.20 per cent are dissolved in 1.25 per cent sulfuric acid, and 26.70 per cent are dissolved by a 1.25 per cent alkali solution. The solubility of the pentosans of non-leguminous plants is quite similar to that of the pentosans of the legumes, but there is enough difference to give misleading results in some cases. In the non-leguminous plants, 24.80 per cent of the pentosans are dissolved by 0.02 N acid and alkali solutions, 26.90 per cent by a 1.25 per cent sulfuric acid solution, and 29.50 per cent by the alkali solution.

Hydrolysis of the pentosans with hot mineral acids may lead to the decomposition of some of the sugars, and one may not be able to account thus for all the pentosan. In studying the composition of pine wood (31), it was found that the H-ion concentration of the sulfite solution must be very carefully adjusted; if it is not of the proper concentration, the cellulose may be attacked, the pentosans may be decomposed, or both may occur. An acid mixture, composed of 80 gm. of sodium acid sulfate and 200 cc. of 0.1 N HCl made up to 1 liter with water, will dissolve all the pentosans without destroying the cellulose. When the acid concentration in this mixture is doubled, the cellulose is slightly attacked and some of the pentoses are decomposed. The glucose is not so readily decomposed. After pine wood has been heated in this stronger mixture at 100°C. for 4 days, 20 per cent of the glucose and 72 to 96 per cent of the various pentoses are decomposed.

Attempts to separate the hemicelluloses, the cellulose, and the lignin of plant material by various chemical treatments have not given accurate results. As an example, an analysis of sunflower seed hulls (46) may be cited. The hulls were extracted with ether, dilute hydrochloric acid, and ammonia, and the residue was treated successively with 5 per cent sodium hydroxide solution, Schweizer's reagent (ammoniacal copper hydroxide), dilute hydrochloric acid, and ammonia. This gave a product containing 2.78 per cent hemicellulose, 6.7 per cent cellulose, and 56.7 per cent lignin. In the various fractions obtained, the pentosans were found to make up a total of 81.4 per cent of the "hemicellulose" fraction, 54.5 per cent of the "cellulose" fraction, and 1.38 per cent of the "lignin" fraction. The products obtained on hydrolysis of plant material are intimately combined in the parent substance; these products should not be considered as absolutely identical with the actual plant constituents (9).

Various formulas have been submitted from time to time to explain the structure of pentosans. One of the oldest formulas (111) represented the pentosan as $(C_5H_8O_4)_n$. Within the last few years (96) the formula $(C_{10}H_{18}O_9)_n$ has been advanced. The basis for this formula lies in the fact that 100 per cent of pentosans can never be recovered when determined by the furfural-phloroglucide method, the purest preparations available yielding only 92 to 96 per cent pentosan; no other sugar except a pentose has ever been isolated from a pentosan.

The usual method of determination of pentosan consists in the distillation

with 12 per cent hydrochloric acid to form furfural; this is precipitated as furfural-phloroglucide by the addition of phloroglucin in a 12 per cent hydrochloric acid solution; the pentosan is then calculated from the amount of precipitate (81). These results are frequently too low because the hydrochloric acid may increase in concentration to about 18 per cent after boiling for some time and bring about the destruction of some of the furfural (115); or various aromatic compounds, especially the tannins, combine with the furfural and prevent it from being distilled over. On the other hand, the results obtained by this method may prove to be too high, since other substances besides pentoses yield furfural, especially the uronic acid derivatives. By determining the uronic acid content of the preparation, one can calculate the amount of furfural due to the uronic groups and the amount due to pentose groups (70).

It was found that the process could be hastened (5) by boiling the furfural-phloroglucide precipitate and filtering immediately rather than letting it stand over night. Since the methylpentosans yield methylfurfural upon distillation, the relative amounts of methylfurfural and furfural produced at various stages of the distillation have been studied (27). Most of the furfural is produced early in the distillation, whereas no methylfurfural is produced until at least 150 cc. has been distilled over. Furfural-phloroglucide and methylfurfural-phloroglucide may be separated on the basis of their solubility in ethyl alcohol, the latter being soluble (34). The formation of hydroxy-methylfurfural (76, 129) causes the results to be too high, as it cannot be separated by solubility in alcohol.

The furfural may also be precipitated with phenylhydrazine (60), fixed by a bisulfite solution or used to reduce copper compounds; it may also be titrated with a standard solution of potassium bromate (81); the formation of compounds with thiobarbituric acid was found (129) to give the most reliable quantitative results.

When pentosans are determined by means of hydrolysis with dilute mineral acids, some of the resulting pentoses may be destroyed (31) by the acid. It becomes necessary, therefore, to keep the H-ion concentration as low as possible, the time of reaction as short as possible, and to avoid high pressures, if accurate results are to be obtained.

It is sufficient to consider at further length the most common pentosan xylan, or "wood gum." Xylan is found abundantly in plant materials, especially in wood, in corn cobs, etc. Methyl-xylan has also been found in nature (42). When xylan is hydrolyzed with 12 per cent hydrochloric acid, 88 per cent of it is changed into sugar in 5 minutes' time, the maximum amount of pentose being formed in 5 to 10 minutes (39, 43). After 10 minutes, the amount of pentose decreases because of its conversion into furfural. When 5 per cent hydrochloric acid was used as the hydrolyzing agent, 90 per cent of the xylan was converted into xylose in 30 minutes. Sulfuric acid hydrolyzed xylan much more slowly than did hydrochloric acid, one hour being necessary to hydrolyze 85 per cent of the xylan with 12 per cent sulfuric acid. Continued action of

the aforementioned acids led to the formation of furfural and later its polymerization and decomposition. It has also been observed (41) that when xylan is heated with 45 parts of 3 per cent nitric acid, 84 per cent of it is hydrolyzed in one hour. This method has a certain advantage, since no furfural is formed in the hydrolytic process. The concentration of nitric acid given in the foregoing will completely dissolve the xylan in 1 to 2 minutes at 100°C. At this stage, part of it is probably in the xylo-dextrin stage. The use of nitric acid as a hydrolyzing agent causes the oxidation of the xylan molecule to tri-hydroxy-glutaric acid, and later to oxalic acid.

Xylan is resistant to the action of alkalies (45); it reacts with acetyl chloride to form xylan-monoacetate, an amorphous powder of the composition $C_5H_7O_3 \cdot C_2H_3O_2$ (111). The xylan-di-acetate is formed when it interacts with acetic anhydride. With benzoylchloride, in the presence of sodium hydroxide, xylan yields a xylan-monobenzoate. Concentrated nitric acid gives a xylan-nitrate. Xylan can be separated from other pentosans, especially from arabans and meta-arabans, by precipitation. Fehling's solution will precipitate all of the xylan from a sodium hydroxide solution (92), while most of the other pentosans remain in solution. In purifying xylan, it is difficult to remove all the free xylose from the xylan molecule proper.

Xylan has been called a polyose (50) in which a reducing group of one xylose molecule is joined with the hydroxyl group of another. The fact that xylan gives mono- and di-acetyl compounds upon acetylation indicates the presence of two free hydroxyl groups. Methylation, hydrolysis, and oxidation of the xylan molecule gave di-methoxy-glutaric acid (50), which led to the suggestion of the following structure for xylan molecule:

$$-OCH_2CH \cdot CH(OH) \cdot CH(OH) \cdot CHOCH_2CH \cdot CH(OH) \cdot CH(OH) \cdot CHO-$$
$$\underline{\qquad O \qquad} \quad\quad \underline{\qquad O \qquad}$$

Schmidt (94, 94a) found that the residual substance obtained by treating plant material with ClO_2 and dilute sodium sulfite solution consists of cellulose and hemicellulose. The latter could be then dissolved out with sodium hydroxide solution and precipitated with alcohol and acid. Two xylans were thus obtained, one of which ("easily-soluble") was removed with dilute (0.04–0.2 per cent) NaOH solution and the other ("difficultly-soluble") was dissolved in more concentrated (5 per cent) NaOH solution.

Of the various other "hemicelluloses," the chemical structure of the pectins has received considerable attention. An extensive literature is available dealing with the chemical nature of this substance (7a). It is sufficient to mention here the fact that pectin of orange peels is considered (24) to consist of an araban and a Ca, Mg salt of pectic acid. The latter gives on hydrolysis several complexes,

$$C_{41}H_{60}O_{36} + 9\ H_2O = 4\ C_6H_{10}O_7 + 2\ CH_3OH + 2\ CH_3 \cdot COOH + C_5H_{10}O_5 + C_6H_{12}O_6$$

| pectic acid | galactu-ronic acid | methyl alcohol | acetic acid | arab-inose | galac-tose |
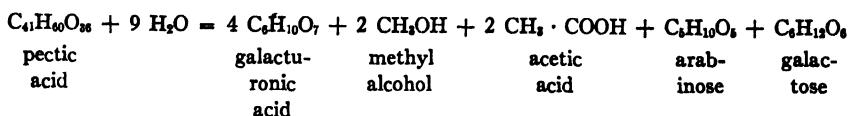
Pectic acid is thus found to be a di-methoxy-di-acetyl-arabino-galacto-tetra-galacturonic acid.

PREPARATION OF CERTAIN CRUDE HEMICELLULOSES

In carrying out the following investigations on the decomposition of specific hemicelluloses by microörganisms, it was necessary to isolate and prepare these polysaccharides in a more or less pure form (107).

Dulse, a seaweed, was the source of the *pentosan* preparation. After thorough washing of the material with cold water, successive extracts with boiling water were made. The extracts were filtered and the filtrates thus obtained were concentrated on a water bath; the pentosan was precipitated from the solution by the addition of 3 volumes of 95 per cent ethyl alcohol. The precipitate was washed with 95 per cent ethyl alcohol and sulfuric ether, and then dried over sulfuric acid in a desiccator at a low temperature.

An analysis of the preparation thus obtained gave the following results:

		per cent
Moisture	..	17 40
Ash		7 00
Total nitrogen		0 57
Pentosan		38 34
Total hemicellulose		60 00

The moisture content was determined by drying to constant weight in an electric oven at 105°, and the ash by ignition. The ordinary Kjeldahl method was used for the determination of total nitrogen. The pentosan was calculated from the furfural produced by distillation of the material with 12 per cent HCl; the furfural was precipitated with phloroglucin. Hydrolysis with 2 per cent hydrochloric acid solution and determination of the resulting reducing sugars gave the total amount of hemicellulose.

The preparation thus obtained was insoluble in cold and hot water. A very fine suspension could be made in hot water; it required considerable time for it to precipitate out again. The preparation did not reduce Fehling's solution. At the boiling point with 2 per cent HCl it went into solution and could then be easily hydrolyzed to simple reducing sugars. It had practically no swelling capacity in water. The ash content of this preparation was noticeably high and the crude protein made up a little over 3 per cent of the total constituents. Although this was called a pentosan preparation, only about 65 per cent of the total hemicellulose was found to be pentosan.

The *mannan* was extracted from salep roots. It was brought into solution with hot water, after thorough washing with cold water. Since the solution could not be filtered because of its viscous nature, the solid particles were centrifuged from the solution. The mannan was then precipitated with 95 per cent ethyl alcohol and the precipitate dried as in the case of the pentosan preparation.

Upon analysis, it gave the following composition:

	per cent
Moisture	. 22 90
Ash	. 1 40
Total nitrogen	0 14
Pentosan	1 82
Total hemicellulose	. 57 48

This preparation was not soluble in cold water and did not reduce Fehling's solution. When it was allowed to become fully imbibed with distilled water, it swelled to three times its dry volume. The ash and total nitrogen contents of this preparation were very low. Only a small portion of the hemicellulose was in the form of pentosan. The reaction with phenylhydrazine showed that the preparation consisted principally of mannan.

The *galactan* was extracted from Irish moss (*Chondrus crispus*), in a manner similar to the above procedures, giving a preparation which analyzed as follows:[2]

	per cent
Moisture	38 60
Ash	18 00
Total nitrogen	0 44
Pentosan	2 18
Total hemicellulose	31 64

It was difficult to remove the residual moisture of this material. This preparation may appear to be low in hemicelluloses, but when the ash and moisture are considered, it becomes obvious that it was as pure as either of the preceding preparations. It was not soluble in cold water and did not reduce Fehling's solution. Upon wetting, it swelled to two and one-half times its dry volume. Haas (30b) concluded that the ash of carragen (*Chondrus crispus*), which cannot be reduced by dialysis and which consists principally of calcium and sulfate, is an integral part of the carragen molecule and is present there as a sulfuric ester.

The *xylan* was extracted from corn cobs. The method used was that of Salkowski as modified by Heuser (84). It consists in extracting the xylan with 5 per cent NaOH solution under 15 pounds pressure for 1 hour and precipitating the xylan with Fehling's solution. The xylan was freed from copper by washing with alcohol saturated with HCl gas. The copper-free compound was washed with ethyl alcohol and ether, and dried in a desiccator over sulfuric acid at a low temperature.

The analysis of the preparation gave the following results:

	per cent
Moisture	5 7
Ash	6 9
Total nitrogen	0 0
Pentosan	88 3
Total hemicellulose	87 7

[2] More purified preparations were later obtained, containing 9 45 per cent moisture and 21 00 per cent ash.

This was by far the purest preparation obtained. Calculated upon the ash- and moisture-free basis, it was made up of 96 per cent pentosan. The reaction with phenyl hydrazine showed that the substance is built up only of xylose anhydrides. It was readily soluble in hot HCl, insoluble in cold water, and only slightly soluble in hot water. It did not reduce Fehling's solution. Upon becoming fully imbibed with distilled water, it attained a volume of approximately 1.5 times its original dry volume. It did not form a gummy mass when placed in cold water, a characteristic property of the aforementioned hexosans studied.

These crude preparations were used in the first studies of decomposition of hemicelluloses by microörganisms, which will be reported in the following contributions. The last three preparations were practically free from uronic acid complexes, hence they can be considered as true hemicelluloses and not as polyuronides.

DECOMPOSITION OF HEMICELLULOSES IN PEAT, IN SOIL, AND IN COMPOSTS

In most of the former investigations on the decomposition of pentosans or of hemicelluloses in general, no attempt has been made to obtain a preparation free from sugars, proteins, cellulose, and lignin. The utilization of hemicellulose by microörganisms was measured by determining its content in the natural material at the beginning and at the end of the decomposition period. It was found, for example (23), that when manure undergoes decomposition, the pentosans are attacked more quickly than the cellulose, whereas toward the end of the decomposition period, more pentosan is left in the compost than in the cellulose. In general pentosans are among those substances which are actively decomposed in the manure pile (121). When sheep manure was allowed to decompose with 100, 200, and 400 per cents moisture, the amounts of hemicellulose left at the end of 192 days were 9.14, 7.31, and 11.62 per cent respectively, compared with 18.46 per cent in the original manure. These results have shown that there is an optimum moisture for the decomposition of the hemicelluloses. Fresh horse manure, on a dry basis, contained 23.52 per cent hemicellulose. When allowed to decompose, the percentage of hemicellulose found after an incubation period of 39, 96, 156, and 290 days, was 22.84, 15.76, 13.36, and 12.67 respectively. When one keeps in mind the fact that a considerable reduction in the weight of dry material has taken place as a result of decomposition, one will readily recognize the rapidity with which these hemicelluloses decompose in compost.

The nature and age of the plant residues have considerable influence upon the rapidity with which the hemicelluloses disappear in the process of decomposition. In the case of rye plants, for example, the rate of decomposition of the pentosans was inversely proportional to the age of the plant (123). In the case of mature rye straw undergoing decomposition in a compost, 77.7 per cent of the pentosans disappeared in two months' time (120), in the presence of available minerals and at an optimum moisture content. The fact that

available nitrogen is necessary in order that the fungi and bacteria can decompose the hemicelluloses was well illustrated. Without additional inorganic nutrients, 15.2 per cent of the hemicellulose was decomposed in 28 days, whereas with added nutrients, the amount decomposed during the same period was 64.7 per cent of the total. In the case of semimature corn stalks, the amounts of hemicelluloses decomposed during the same period in the composts, without and with inorganic nutrient salts were 50.6 per cent and 81.4 per cent respectively. Mature rye straw composts containing 2,139 gm. of hemicellulose lost the following amounts within a period of 290 days: untreated compost, 868.3 gm.; calcium carbonate added, 706.2 gm.; diammonium phosphate and potassium chloride added, 1,407.6 gm.; a combination of the three salts, 1565.5 gm.

In the composting of oak leaves under aerobic and anaerobic conditions (125), in the presence of diammonium phosphate and calcium carbonate, large quantities of hemicellulose were decomposed, as is shown in table 3.

TABLE 3

Decomposition of oak leaves under aerobic and anaerobic conditions

CONDITIONS	Original leaves	AMOUNTS OF HEMICELLULOSE PRESENT			
		After 6 months		After 12 months	
		No CaCO₃	With CaCO₃	No Ca	With Ca
	gm	*gm.*	*gm*	*gm.*	*gm.*
Aerobic..	25 00	6 62	6 62	5.85	5 55
Anaerobic.......	25 00	22 95	22 72	12.81	7.45

According to Rege (85), two factors influence the rapidity of decomposition of plant material, in the presence of sufficient available nitrogen: the "energy" factor, which he associated with the pentosans, and the "inhibitory" factor which he believed to be the lignin fraction; he suggested that the ratio between these two factors can enable one to predict the rapidity of decomposition of the given material. This assumption is totally unjustified, as shown recently by Norman (71) and previously by Egorov (24) and in this laboratory (109), where the hemicelluloses were found to decompose rapidly during the first few days, and later only slowly.

This was found to be due to three factors: (a) the non-homogeneous nature of the hemicelluloses, some of which are decomposed much more readily than others (b) the possible production of some intermediate substance which yields furfuraldehyde (70), and (c) the synthesis of new hemicelluloses by microorganisms. The reason for the second assumption was found (71) in the small loss of uronic acid in the process of decomposition of straw. The third factor was established in former contributions from this laboratory on the synthesizing activities of microörganisms.

This phenomenon can further be illustrated when one compares the relative hemicellulose and cellulose content of a lowmoor peat (119, 124); although the plants from which the peat originated contained a much higher cellulose than hemicellulose content, the peat itself was practically free from cellulose, but it still contained 6 to 12 per cent hemicellulose.   von Feilitzen and Tollens (25) found that the pentosan content of peat diminishes with depth, 6.26 to 12.75 per cent of pentosan being found in the surface layers.   Since more hemicellulose was decomposed in the older peat horizons, an attempt has been made to determine the age of the horizon by the amount of hemicelluloses present (115).   The same is true of the organic matter layer in forest soils, which may contain 12 to 18 per cent hemicellulose and only 2.5 to 10 per cent cellulose, whereas the reverse ratio between these two plant constituents is found in the undecomposed plant residues (125).

König and associates (51) have shown that the hemicelluloses added to the soil in the manure decompose more readily than the total carbon complexes (due to the resistance of the lignin) and, after a period of one year, nearly all the pentosans have disappeared.   At least some of the hemicelluloses in the organic matter of mineral soils is of microbial origin (30).

Various fungi (69) and bacteria (4) are capable of producing cytases or enzymes capable of hydrolyzing hemicelluloses.   A detailed discussion of the decomposition of various hemicelluloses by pure cultures of microörganisms will be presented later.

SUMMARY

1. Attention is called to the confused state of our present knowledge of the hemicelluloses, which form an important group of chemical constituents of plants and microörganisms.

2. The chemistry of hemicelluloses, methods of analysis, and the occurrence of these carbohydrates in nature are discussed in detail.

3. A general review is presented of the decomposition of hemicelluloses by microörganisms, when plant materials undergo decomposition in manure compost and in soil.

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ON THE DECOMPOSITION OF HEMICELLULOSES BY MICRO-ORGANISMS: II. DECOMPOSITION OF HEMICELLULOSES BY FUNGI AND ACTINOMYCES[1]

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It has been variously shown that a large number of fungi are capable of decomposing different hemicelluloses, using these carbohydrates as sources of energy and for the building up of microbial cell substance. The chemical processes involved in the decomposition of the various hemicelluloses, especially the nature of the products formed, are still little known. Certain species of *Aspergillus*, for example, have been shown (1) to be able to produce enzymes hydrolyzing mannans and galactans, when grown in solutions containing the respective sugars. Representatives of the genera *Mucor, Rhizopus, Botrytis, Cladosporium, Thamnidium, Penicillium, Trichothecium*, etc. were found by Schellenberg (7) capable of attacking various hemicelluloses. A very specific difference exists, however, in the ability of the different microörganisms to decompose cellulose and hemicelluloses. The Mucorales, for example, are unable as a group to attack pure cellulose but are able to utilize very readily a number of hemicelluloses. Hemicelluloses of different origin differ markedly in this connection, due probably to a difference in chemical nature. The hemicellulose of the endosperm of dates, which gives on hydrolysis with acids galactose and mannose, was found to be very resistant to decomposition by microörganisms; out of 16 species of fungi tested, only 2 (*Penicillium* sp. and *Trichothecium roseum*) were able to attack this carbohydrate. On the other hand, the hemicellulose of *Lupinus hirsutus*, which gives on hydrolysis with dilute acids galactose and arabinose, was readily decomposed by a number of fungi, including species of *Mucor, Rhizopus, Botrytis*, and *Cladosporium*. According to Otto (4), species of *Mycogone, Stemphylium, Starchybotrys*, and various other fungi are also capable of attacking the hemicellulose of the date endosperm.

Fungi can attack readily xylans and other pentosans (6, 9). The majority of investigations dealing with the decomposition of pentosans by microorganisms were usually carried out by inoculating a nutrient medium containing a certain plant material with a specific organism, incubating for a certain period of time and measuring the rapidity of the disappearance of the pen-

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tosan by the production of furfural on distillation with 12 per cent hydrochloric acid. Because of the fact, however, that furfural is produced under these conditions both from pentosans and from uronic acid complexes, one can readily recognize that this method of measurement gives only a very imperfect idea of the nature of the substance that has undergone decomposition. It was found (8), for example, that the source of the pentosan has an influence upon the rapidity of its decomposition by specific organisms. About 50 per cent of the pentosan in corn forage was destroyed by pure cultures of fungi in 100 days, and only 35 per cent of the pentosan in the rye straw in 300 days. This difference may be due not so much to the accompanying complexes in the different plant materials, but largely to the different chemical nature of the hemicelluloses in these materials. *Asp. fumigatus* was most active in decomposing the pentosan and *Rhizopus nigricans* least; the former is known to be a cellulose-decomposing organism, whereas the latter cannot attack cellulose. The ability of certain specific microörganisms to bring about the decomposition of specific hemicelluloses, such as pectins, has an important application in certain plant diseases (2), in the preparation of pectin from fruits (5), in the processes of retting of flax, and in the decomposition of plant and microbial residues in composts and in soil.

In their relation to hemicelluloses, microörganisms possess a definite specificity, more so than in the case of the microbes attacking cellulose and proteins, due both to the specific physiology of the different organisms and to the specific chemical nature of the different hemicelluloses. Some microörganisms will attack hemicelluloses more readily during the early stages of decomposition of a plant material, and later attack the cellulose; however, some hemicelluloses are more resistant to the action of microörganisms than is cellulose (11). Certain species of fungi, like yeasts, are entirely unable to attack hemicelluloses (3, 10).

The total number of fungi capable of decomposing hemicelluloses is considerably greater than the number attacking pure cellulose. This ability is not limited to any one group, but is the property of numerous organisms belonging to the Phycomycetes, Ascomycetes, Fungi Imperfecti, and Basidiomycetes. The information concerning the ability of these various fungi to decompose specific hemicelluloses is still very limited. Still less is known of the behavior of actinomyces in this respect.

In the following investigations, an attempt has been made to study the decomposition of certain purified hemicellulose preparations, which have been freed from sugars, cellulose, and lignin. The methods of obtaining these "hemicelluloses" have been described previously (12). The fungi used for these studies were selected from the laboratory stock cultures. The actinomyces, however, were freshly isolated from a fertile soil in which the specific hemicelluloses were undergoing active decomposition. No attempt has been made to identify and describe the various species of actinomyces thus isolated, since they were considered in these experiments more as types representing

this particular genus in its ability to decompose hemicelluloses; only active organisms have been selected for this purpose.

DECOMPOSITION OF MANNAN

The decomposition of mannan, as well as that of the other hemicellulose preparations, was studied both in liquid and in sand culture media. In making up these media, the following nutrients were added to 100-cc. portions of solution, or to 100-gm. portions of sand containing 20 cc. water:

	gm
$(NH_4)_2HPO_4$	0 050
K_2HPO_4	0 050
$MgSO_4$	0 010
NaCl	0 010
$CaCl_2$	0 010
$FeSO_4$	0 001
Mannan	0 500

These portions of liquid or sand media containing the nutrients were placed in a series of flasks, sterilized, inoculated with the various pure cultures of the organisms, and incubated at 27 to 28°C. for a period of 42 days.

At the end of the incubation period the contents of the flasks were analyzed for total hemicellulose and for ammonia nitrogen. Instead of the hemicellulose being boiled with acid under a reflux condenser for 5 hours, the hydrolysis was effected by autoclaving with 2 per cent HCl under 15 pounds pressure for 30 minutes. This method of hydrolysis can be employed only when there are no other polysaccharides present which would give reducing sugars upon hydrolysis with dilute acids under pressure. Ammonia determinations were made by distilling an aliquot portion of the culture with magnesium oxide.

The decomposition of the mannan in the sand medium by pure cultures of fungi is shown in table 1. All of the fungi tested were found to be able to decompose the mannan very actively. The maximum decomposition was noted in the case of the *Rhizopus*, which brought about the destruction of 95 per cent of the mannan. The *Penicillium* was found to be the least active of the fungi tested, decomposing in this experiment only 76 per cent of the mannan present in the medium. When one compares the amount of nitrogen required by the fungi to bring about the decomposition of the mannan, one finds that, on an average, 35.5 parts of mannan were decomposed per one part of inorganic nitrogen assimilated. In addition to bringing about the greatest decomposition of the mannan, the *Rhizopus* was also the organism that required the least nitrogen, 49.2 parts of the mannan being decomposed per one part of nitrogen assimilated. It is of considerable interest and possibly of practical importance to call attention to the fact that the three representative genera of Phycomycetes, namely *Zygorhynchus, Cunninghamella,* and *Rhizopus,* which are known (13) to be unable to decompose true cellulose, proved to be most active in the decomposition of the hemicellulose mannan. These three

organisms were not only as active in decomposing the mannan as the true cellulose decomposing fungi, namely *Trichoderma, Humicola*, and *Asp. fumigatus*, but they required less nitrogen for this decomposition than the other organisms, at least in sand media.

TABLE 1

The decomposition of mannan in sand medium by pure cultures of fungi

ORGANISM	MANNAN		AMMONIA-NITROGEN		PARTS OF MAN-NAN DECOM-POSED PER UNIT OF NITROGEN ASSIMILATED
	Found	Decomposed	Found	Assimilated	
	$m_L m$	*mgm*	*mgm*	*mgm*	
Control medium	305 5		9 6		. .
Zygorhynchus	25 9	279 6	2 9	6 7	41 7
Cunninghamella	30 6	274 9	2 3	7 3	37 7
Rhizopus	15 1	290 4	3 7	5 9	49 2
Penicillium	71 8	233 7	2 4	7 2	32 5
Trichoderma	20 5	285 0	0 8	8 8	32 4
Humicola	55 6	249 9	1 8	7 8	32 0
Asp niger	59 9	245 6	1 9	7 7	31 9
Asp fumigatus	28 4	277 1	1 8	7 8	35 5

TABLE 2

Decomposition of mannan in solution medium by pure cultures of fungi

ORGANISM	pH	MANNAN		AMMONIA NITROGEN		PARTS OF MAN NAN DECOM POSED PER UNIT OF NITROGEN ASSIMILATED
		Found	Decomposed	Found	Assimilated	
		mgm	*mgm*	$m_L m$	*mgm*	
Control	5 4	382 5		9 6		
Zygorhynchus	5 0	255 2	127 3	6 0	3 6	32 6
Cunninghamella	5 2	21 6	360 9	1 0	8 6	41 9
Rhizopus	4 8	14 9	367 6	0 6	9 0	40 8
Penicillium	5 2	8 1	374 4	2 8	6 8	55 1
Trichoderma	5 2	270 5	112 0	5 2	4 4	25 4
Humicola	5 0	21 6	360 9	1 2	8 4	42 8
Asp niger	5 0	21 6	360 9	0 6	9 0	40 1
Asp fumigatus	5 2	17 1	365 4	0 8	8 8	40 3

In order to compare the influence of the nature of the medium upon the rapidity and nature of decomposition of mannan by various fungi, the same organisms were inoculated into a sterile solution containing mannan and inorganic nutrient salts. The results obtained in this experiment are reported in table 2. Colorimetric determinations of the hydrogen-ion concentration of the medium as expressed by their pH value, were also made in all the cul-

tures. The increase in the H-ion concentration in the inoculated flasks indicates the formation of organic acids during the decomposition process. The results are not in exact conformity with those obtained in the sand medium. *Penicillium*, for example, which decomposed the least amount of mannan in the sand medium, brought about the greatest decomposition in the solution me-

TABLE 3

The decomposition of mannan in sand medium by pure cultures of soil actinomyces

ORGANISM	MANNAN		AMMONIA NITROGEN		PARTS OF MANNAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
	Found	Decomposed	Found	Assimilated	
	mgm	mgm	mgm	mgm	
Control	305 5		9 6		
Actinomyces 26	31 3	274 2	1 2	8 4	32 6
Actinomyces 40	15 1	290 4	4 1	5 5	52 8
Actinomyces 48	25 9	279 6	5 8	3 8	73 6
Actinomyces 50	17 8	287 7	3 7	5 9	48 8
Actinomyces 54	26 1	279 4	4 3	5 3	52 7
Actinomyces 33	23 9	281 6	4 7	4 9	57 5
Actinomyces 51	44 1	261 4	3 2	6 4	40 8
Actinomyces 76	32 9	272 6	2 5	7 1	38 4

TABLE 4

Decomposition of mannan in solution medium by pure cultures of soil actinomyces

ORGANISM	pH	MANNAN		AMMONIA NITROGEN		PARTS OF MANNAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
		Found	Decomposed	Found	Assimilated	
		mgm	mgm	mgm	mgm	
Control	5 4	382 5				
Actinomyces 26	5 2	203 9	178 6	4 4	5 2	34 4
Actinomyces 40	5 2	28 4	354 1	2 8	6 8	52 1
Actinomyces 48	5 2	127 8	254 7	2 2	7 4	34 4
Actinomyces 50	5 2	194 0	188 5	3 4	6 2	30 4
Actinomyces 54	5 2	208 8	173 7	5 8	3 8	45 7
Actinomyces 33	5 0	164 3	218 2	1 2	8 4	26 0
Actinomyces 51	5 2	99 0	283 5	0 2	9 4	30 2
Actinomyces 76	5 2	176 9	205 6	1 6	8 0	25 7

dium, 97.9 per cent of the mannan originally present having disappeared during the period of decomposition The ratio between the decomposition of the hemicellulose and the nitrogen utilization was wider in the solution cultures than in the sand cultures.

The following two experiments deal with the decomposition of the mannan by a series of actinomyces. The action of these organisms upon the hemi-

cellulose in a sand medium is reported in table 3. The process of decomposition was carried out rapidly by the actinomyces as well, although these organisms are usually reputed to be slow growing; *Actinomyces 40* brought about the greatest decomposition, 95 per cent of the mannan having disappeared in the given period of time. The lowest amount of decomposition was noted in the case of *Actinomyces 51*, but even in this case 86 per cent of the mannan originally present in the medium has disappeared. The utilization of nitrogen in the decomposition of this polysaccharide by actinomyces shows more variation than in the case of the fungi: the average ratio of mannan decomposed to the inorganic nitrogen assimilated was 47.2 with 73.6 as the maximum and 32 6 as the minimum, this ratio being wider than in the case of the fungi.

The results of the decomposition of mannan by pure cultures of actinomyces in a liquid medium are given in table 4. The changes in pH in these cultures were not very great. Only in one case, namely with the *Actinomyces 33*, was

TABLE 5

The decomposition of mannan in salep root by pure cultures of fungi and actinomyces

ORGANISM	pH	MANNAN		AMMONIA NITROGEN		PARTS OF MANNAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
		Found	Decomposed	Found	Assimilated	
		mkm	*mgm*	*mgm*	*mgm*	
Control	7 0	660 4		62 0		
Zygorhynchus	6 4	224 6	435 8	57 6	4 4	99 0
Rhizopus	6 8	47 5	612 9	48 4	13 6	45 0
Penicillium	7 0	39 4	621 0	46 4	15 6	39 8
Trichoderma	6 6	340 7	319 7	56 4	5 6	57 1
Humicola	7 0	83 2	577 4	54 0	8 0	72 2
Actinomyces	6 4	44 8	615 6	48 0	14 0	44 0

the pH value lowered by 0.4. The average amount of mannan decomposed by actinomyces in solution culture was 67 per cent of the total. However, the *Actinomyces 40* decomposed 92 per cent of the total mannan present in the medium. On the average, one part of inorganic nitrogen was assimilated by the actinomyces for every 34.8 parts of mannan destroyed in liquid medium. These organisms decomposed considerably larger amounts of hemicellulose per unit of inorganic nitrogen assimilated in sand than in solution media, or a larger quantity of nitrogen is transformed from an inorganic into an organic form by the same organism when it grows in a liquid medium than in a fully aerated sand medium.

The results presented here prove beyond any doubt that when mannans are applied to soil in the complex plant materials, they soon disappear, if conditions for their decomposition are favorable. The above investigations were carried out by the use of purified mannan. In order to determine whether

or not the extraction and preparation of the mannan had any influence upon the nature and rapidity of its decomposition, the utilization of mannan in its natural state, namely in the form of salep root by microòrganisms, was studied. These studies were carried out in liquid medium only.   The results, reported in table 5, show that from 48.4 to 94.0 per cent of the mannan in the natural product was decomposed by the fungi; in other words, the decomposition is as rapid as that of the purified preparation.   The actinomyces decomposed 93.2 per cent of the mannan added to the medium.   The nitrogen consumption by the organisms bringing about the decomposition of the mannan in the salep root is considerably less than in the case of the purified mannan preparation, 62.6 units of the mannan being destroyed per unit of nitrogen assimilated, in the case of the fungi, and 44 parts in the case of the actinomyces.   The nitrogen utilization value in this experiment should, of course, not be considered as

TABLE 6

The decomposition of xylan in sand medium by pure cultures of fungi

ORGANISM	XYLAN		AMMONIA NITROGEN		PARTS OF XYLAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
	Found	Decom posed	Found	Assimi lated	
	mgm	*mgm*	*mgm*	*mgm*	
Control	152 0	.	8 6		
Zygorhynchus	107 1	44 9	6 1	2 5	18 0
Cunninghamella	67 0	85 0	3 4	5 2	16 3
Rhizopus	79 2	72 8			
Penicillium	79 2	72 8	3 9	4 7	15 2
Trichoderma	65 7	86 3	2 8	5 8	14 9
Humicola	88 2	63 8	3 4	5 2	12 3
Asp fumigatus	52 9	99 1	2 8	5 6	22 5

exact as in the experiments with the purified mannan, because in the former instance the organisms had at their disposal some of the nitrogen present in the salep root itself, which they used in preference to the added inorganic nitrogen.

DECOMPOSITION OF XYLAN BY MICROORGANISMS

The experiments dealing with the decomposition of the xylan preparation were carried out in the same manner as outlined for the decomposition of the mannan.   The flasks were inoculated with organisms which had shown, in previous experiments (not reported here), that they were capable of decomposing xylan.   The incubation period was 6 weeks at a temperature of 27 to 28°C.   The methods of quantitative analysis were also the same as those employed in the previous experiments.

All the fungi tested (table 6) decomposed the xylan, but to a varying extent.

The range of decomposition was 29 5 and 83.0 per cent of the xylan in the sand medium. *Aspergillus fumigatus* brought about the maximum destruction of the xylan, whereas *Zygorhynchus* attacked this carbohydrate to the least extent. In bringing about this process, the fungi consumed a large amount of nitrogen, the average being about one unit of nitrogen consumed for every 16 7 parts of xylan decomposed. The reason for this large consumption of

TABLE 7

The decomposition of xylan in solution medium by pure cultures of fungi

ORGANISM	pH	XYLAN		AMMONIA NITROGEN		PARTS OF XYLAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
		Found	Decom posed	Found	Assimi lated	
		mgm	*mgm*	*mgm*	*mgm*	
Control	6 2	287 3		8 6		
Zygorhynchus	6 2	235 8	51 5			
Cunninghamella	6 0	132 6	154 7	3 2	5 4	28 7
Rhizopus	6 2	232 2	55 1	6 6	2 0	27 6
Penicillium	6 2	129 0	158 3	5 0	3 0	52 8
Trichoderma	6 2	126 0	161 3	2 6	6 0	26 9
Humicola	6 2	92 4	184 9	3 4	5 2	35 6
Asp niger	5 8	129 0	158 3	1 6	7 0	22 6
Asp fumigatus	5 8	110 4	176 9	1 2	7 4	23 9

TABLE 8

The decomposition of xylan in sand medium by pure cultures of actinomyces

ORGANISM	XYLAN		AMMONIA NITROGEN		PARTS OF XYLAN DECOMPO ED PER UNIT OF NITROGEN ASSIMILATED
	Found	Decom posed	Found	Assimi lated	
	mgm	*mgm*	*mgm*	*mₗm*	
Control	152 0		8 6		
Actinomyces 26	38 7	133 3	2 3	6 3	18 0
Actinomyces 40	25 2	126 8	3 3	5 3	23 9
Actinomyces 50	29 7	122 3	2 2	6 4	19 1

nitrogen with xylan as a source of energy in the sand medium remains to be determined.

The decomposition of the xylan in solution medium by pure cultures of fungi is shown in table 7. The greatest decomposition was brought about by *Humicola* and *Asp. fumigatus*, which destroyed 64·and 62 per cent of the xylan respectively in 6 weeks. The *Rhizopus* and *Zygorhynchus* brought about the least decomposition, so that the average amount of xylan destroyed by the various fungi was 49.2 per cent. In the case of the liquid medium 27.6 parts of xylan were decomposed for every unit of nitrogen assimilated.

The decomposition of xylan by actinomyces is reported in tables 8 and 9. The results c decomposition in the sand medium, where only three strains were employ(d, show that these organisms are capable of decomposing xylans very rapidly. on the average, 79.5 per cent of the xylan present in the medium disappeared in 6 weeks. The fact that 20.3 parts of the xylan were decomposed

TABLE 9

The decomposition of xylan in solution medium by pure cultures of actinomyces

ORGANISM	XYLAN		AMMONIA NITROGEN		PARTS OF XYLAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
	Found	Decom posed	Found	Assimi lated	
	mgm	*mgm*	*mgm*	*mgm*	
Control	287 3		8 6		
Actinomyces 26	178 9	108 4	5 2	3 4	31 9
Actinomyces 40	186 6	100 7			
Actinomyces 48	186 6	100 7	4 4	4 2	24 0
Actinomyces 50	235 8	51 5	7 8	0 8	64 4

TABLE 10

The decomposition of xylan in corn cobs in solution medium by pure cultures of fungi and actinomyces

ORGANISM	pH	XYLAN		AMMONIA NITROGEN		PARTS OF XYLAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
		Found	Decom posed	Found	Assimi lated	
		mgm	*mgm*	*mgm*	*mgm*	
Control	6 8	405 5		30 0		
Rhizopus	6 6	187 9	237 6			
Penicillium	6 6	223 6	181 9	23 6	6 4	28 4
Trichoderma	6 6	331 0	74 5	28 0	2 0	37 3
Humicola	6 5	299 7	105 8	25 6	4 4	24 1
Cunninghamella	6 6	191 2	214 3	28 4	1 6	
Asp niger	6 5	302 9	102 6	28 0	2 0	51 3
Asp fumigatus	6 5	187 5	218 0	25 2	4 8	45 4
Actinomyces 26	6 6	372 1	33 4	28 4	1 6	20 9
Actinomyces 50	6 5	362 9	42 6	27 6	2 4	17 8
Actinomyces 40	6 5	306 2	98 3			

for every unit of nitrogen assimilated shows an extensive energy consumption just as in the case of the fungi. In liquid medium (table 9) the actinomyces proved to be much less efficient, because of unfavorable cultural conditions. On the average, 31.4 per cent of the xylan originally present in the medium was decomposed; one part of nitrogen was assimilated for every 40.1 parts of xylan destroyed.

In order to study the decomposition of the xylan in the natural state, ground corn cobs were employed. Xylan makes up about 30 per cent of the total constituents of the dry corn cobs. Although the microörganisms were capable of attacking the other constituents in the corn cobs as well, only the transformation of the xylan portion was taken into consideration. The studies were carried out in a manner similar to the previous experiments, using a solution medium. The results are given in table 10. On the average, the fungi decomposed 43.1 per cent of the xylan originally present in the corn cobs, whereas the actinomyces brought about the disappearance of only 14.3 per cent of the xylan. These results show definitely that a certain chemical complex may be decomposed by microörganisms in a different manner in a purified condition than when present in the natural state in the plant material. Whether this is due to the influence of the other organic complexes in the plant upon the

TABLE 11

The decomposition of galactan in sand medium by pure cultures of fungi

ORGANISM	GALACTAN		AMMONIA-NITROGEN		PARTS OF GALACTAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
	Found	Decomposed	Found	Assimilated	
	mgm	*mgm*	*mgm*	*mgm*	
Control	170 2		9 3		
Zygorhynchus	127 8	42 4	7 9	1 4	30 3
Cunninghamella	127 8	42 4	8 7	0 6	70 7
Rhizopus	142 2	28 0	7 6	1 7	16 5
Penicillium .	122 4	47 8	8 7	0 6	79 6
Trichoderma 27	108 9	61 3	7 7	1 6	38 3
Humicola	86 4	83 8			
Asp. niger	99 0	71 2	7 6	1 7	41 9
Asp fumigatus	108 9	61 3	7 9	1 4	43 8

decomposition of the specific complex, or due to its chemical or possibly physical modification in the process of preparation and purification remains to be determined. The limitations of nitrogen utilization studies when impure preparations are employed being considered, the fungi brought about the decomposition of 34.5 parts of xylan per unit of nitrogen assimilated, and the actinomyces, 19.4 parts.

DECOMPOSITION OF GALACTAN BY MICROORGANISMS

The decomposition of galactan by various microörganisms was carried out in a manner similar to the studies of mannan and xylan decomposition. The galactan was found to be considerably more resistant to attack by microorganisms than the other two carbohydrates. There was also observed considerably greater variation among the various organisms in their ability to decompose the galactan. The decomposition of galactan in the sand medium

by pure cultures of fungi is reported in table 11. The results show that, on the average, 33.3 per cent of the galactan disappeared as a result of the activities of the fungi. The amount of galactan decomposed per unit of nitrogen assimilated is rather variable, the average value obtained being about 35.

The decomposition of galactan in solution media by pure cultures of fungi (table 12) is quite similar to that in sand media. The nitrogen utilization in this experiment could not be computed with any degree of accuracy because

TABLE 12

The decomposition of galactan in solution medium by pure cultures of fungi

ORGANISM	pH	GALACTAN		AMMONIA-NITROGEN	
		Found	Decom-posed	Found	Assimi-lated
		mgm	*mgm*	*mgm*	*mgm*
Control	7 2	306 9		9 3	
Zygorhynchus	7 6	218 7	88 2	9 0	0 3
Rhizopus	7 4	202 5	104 4	9 0	0 3
Penicillium	7 4	202 5	104 4	8 7	0 6
Cunninghamella	7 6	261 9	45 0	9 3	
Trichoderma	7 4	192 6	114 3	8 7	0 6
Humicola	7 5	208 8	98 1	9 3	
Asp. niger	7 4	192 6	114 3	8 7	0 6
Asp. fumigatus	7 2	192 6	114 3	9 0	0 3

TABLE 13

The decomposition of galactan in sand medium by pure cultures of soil actinomyces

ORGANISM	GALACTAN		AMMONIA-NITROGEN		PARTS OF GALACTAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
	Found	Decom-posed	Found	Assimi-lated	
	mgm	*mgm*	*mgm*	*mgm*	
Control .	170 2		9 3		. .
Actinomyces 26	119 7	50 5	8 1	1 2	42 0
Actinomyces 33	99 0	71 2	8 3	1 0	71 2
Actinomyces 35	135 9	34 3	6 9	2 4	14 7
Actinomyces 40	97 2	73 0	7 4	1 9	38 4
Actinomyces 50	69 3	100 9	7 8	1 5	67 3

of the inconsistent results obtained. In view of the fact that the galactan preparation contained a certain amount of combined nitrogen, which some of the organisms at least could utilize, the exact rôle played by the inorganic nitrogen under these conditions in favoring decomposition could of course not be ascertained. There was an average decomposition of about 30 per cent of the galactan present in the medium.

The destruction of the galactan by actinomyces in sand culture is given in table 13. It has been observed in preliminary experiments that the galactan

supports an active flora of actinomyces in the soil, and one might, therefore, expect that this group of soil microorganisms would decompose a considerable part of the galactan. The actinomyces employed in this experiment actually brought about the disappearance of about 38.8 per cent of the galactan originally present in the medium. These organisms were able to decompose, on

TABLE 14

The decomposition of galactan in solution medium by pure cultures of soil actinomyces

ORGANISM	pH	GALACTAN		AMMONIA NITROGEN	
		Found	Decom posed	Found	Assimi lated
		mgm	*mgm*	*mgm*	*mgm*
Control	7 2	306 9		9 3	
Actinomyces 26	7 5	218 7	88 2	9 7	
Actinomyces 33	7 4	202 5	104 2	9 7	
Actinomyces 35	7 3	258 3	48 6	9 7	
Actinomyces 40	7 4	216 9	90 0	9 0	0 3
Actinomyces 50	6 6	286 2	20 7	8 3	1 0

TABLE 15

The decomposition of galactan in Irish moss in solution by pure cultures of microorganisms

ORGANISM	GALACTAN		AMMONIA NITROGEN		PARTS OF GALACTAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
	Found	Decom posed	Found	Assimi lpted	
	mgm	*mgm*	*mgm*	*mgm*	
Control	382 3		50 0		
Zygorhynchus	265 7	116 6	43 2	6 8	17 1
Rhizopus	259 7	122 6	46 0	4 0	30 7
Penicillium	263 0	119 3	43 2	6 8	17 6
Cunninghamella	244 6	137 7	43 2	6 8	20 3
Trichoderma	265 7	116 6	43 6	6 4	18 2
Humicola	271 6	110 7	46 8	3 2	34 6
Asp niger 7	281 3	101 0	44 0	6 0	16 8
Asp fumigatus	271 6	110 7	48 4	1 6	69 2
Actinomyces 26	263 0	119 3	47 6	2 4	49 7
Actinomyces 33	268 9	113 4	48 8	1 2	94 5
Actinomyces 35	287 3	95 0	48 0	2 0	47 5
Actinomyces 40	253 8	128 5	46 8	3 2	40 2
Actinomyces 50	241 9	140 4	44 8	5 2	27 0

the average, 46.7 parts of galactan for every unit of nitrogen assimilated in the synthesis of their protoplasm. Here again, it is difficult to tell how much the utilization of the organic nitrogen in the galactan preparation modified the consumption of inorganic nitrogen for the synthesis of the microbial cell substance.

The decomposition of galactan in the solution medium by actinomyces is shown in table 14.   The results are very variable, similar to those noted in the decomposition of the galactan by the fungi.   About 22.9 per cent of the total galactan in the medium was destroyed by the actinomyces.   The utilization by the organisms, under these conditions, of the organic nitrogenous constituents of the galactan preparation is indicated by the fact that in certain cultures there was more ammonia at the end of the experiment than at the beginning.   The actinomyces are known to be organisms capable of attacking

TABLE 16

Evolution of carbon dioxide in the decomposition of mannan by pure cultures of fungi

CO_2 as mgm C

ORGANISM	DAYS OF INCUBATION						
	2	4	6	11	18	28	42
	mgm	*mgm*	*mgm*	*mgm*	*mgm*	*mgm*	*mgm*
Control	0 1	0 3	0 7	2 0	3 5	4 0	5 2
Zygorhynchus	15 1	29 3	39 7	49 9	55 7	62 1	72 0
Rhizopus	21 0	30 6	51 8	60 2	63 3	66 0	69 1
Penicillium	9 4	27 0	50 4	60 2	63 9	67 4	70 3
Trichoderma	15 4	34 0	44 2	50 1	58 4	66 5	71 0
Humicola	17 4	37 1	55 7	61 2	64 8	67 0	70 2

TABLE 17

Evolution of carbon dioxide in the decomposition of mannan in sand medium by pure cultures of soil actinomyces

CO_2 as mgm C

ORGANISM	DAYS OF INCUBATION						
	2	4	6	11	18	28	42
	mgm	*mgm*	*mgm*	*mgm*	*mgm*	*mgm*	*mgm*
Control	0 1	0 3	0 7	2 0	3 5	4 0	5 2
Actinomyces 26	11 3	17 5	29 9	43 4	55 9	71 5	91 3
Actinomyces 40	15 1	31 8	47 1	62 2	73 6	80 4	85 8
Actinomyces 48	5 9	13 2	35 5	72 1	84 4	89 6	95 9
Actinomyces 50	11 3	30 2	43 3	64 9	79 8	86 0	89 9

organic nitrogenous complexes as sources of energy in preference even to readily available carbohydrates.

The decomposition of the galactan in the unmodified plant material, namely the Irish moss, was also studied.   It is to be remembered of course that this plant material contained other organic complexes in addition to the galactan, which were undergoing decomposition.   This would influence particularly the nitrogen consumption by the microorganisms.   However, only the decomposition of the galactans was considered in this experiment.   The results

presented in table 15 bring out again the resistance of the galactan in the natural state towards decomposition by various microörganisms. The fungi

TABLE 18

Evolution of carbon dioxide in the decomposition of mannan in salep root by microorganisms

CO_2 as mgm C

ORGANISM	DAYS OF INCUBATION						
	8	11	13	15	18	24	30
	mgm	*mgm*	*mgm*	*mgm*	*mgm*	*mgm*	*mgm*
Control	1 0	1 5	2 1	2 8	3 5	4 0	4 5
Zygorhynchus	14 7	25 4	30 8	34 1	37 7	44 0	48 6
Rhizopus	32 5	59 3	76 1	88 9	97 5	109 9	117 7
Penicillium	14 3	37 1	52 3	63 4	76 8	93 6	104 1
Trichoderma	12 4	33 8	51 3	61 1	66 8	75 3	78 3
Actinomyces 26	5 8	23 3	44 8	61 0	74 2	95 7	102 7

TABLE 19

Evolution of carbon dioxide from xylan by pure cultures of fungi

CO_2 as mgm C

ORGANISM	DAYS OF INCUBATION				
	4	6	18	28	42
	mgm	*mgm*	*mgm*	*mgm*	*mgm*
Control .	0 3	0 8	2 5	3 8	5 1
Zygorhynchus	2 0	2 4	4 1	6 0	10 1
Rhizopus	2 8	3 9	7 0	10 3	13 5
Penicillium	9 7	24 7	39 4	42 1	46 4
Trichoderma	14 4	29 5	43 4	46 7	48 7
Humicola	19 2	30 4	39 6	42 3	47 3

TABLE 20

Evolution of carbon dioxide from xylan by pure cultures of actinomyces

CO_2 as mgm. C

ORGANISM	DAYS OF INCUBATION				
	4	6	18	28	42
	mgm	*mgm*	*mgm*	*mgm*	*mgm*
Control	0 3	0 8	2 5	3 8	5 1
Actinomyces 26	12 8	25 6	48 1	56 8	65 6
Actinomyces 40	17 7	32 4	52 3	60 8	67 8
Actinomyces 50	0 4	2 1	18 5	42 0	52 4

decomposed only 31.0 per cent of the hemicellulose in a period of 6 weeks and the actinomyces 31.2 per cent. It is interesting to note two phenomena brought out in this experiment; namely, the fact that the active cellulose-

decomposing fungi *Trichoderma* and *Asp. fumigatus* decomposed no more galactan, in fact even less, than the non-cellulose decomposing Phycomycetes; the abundant decomposition of galactan by actinomyces confirms the previous observations concerning the use of purified preparations. The fungi and actinomyces assimilated one part of nitrogen for every 27.9 and 51.8 parts of galactan decomposed respectively. This difference in ratio is due both to the greater nitrogen consumption by the fungi and to greater ability to utilize the organic nitrogen in the moss by the actinomyces.
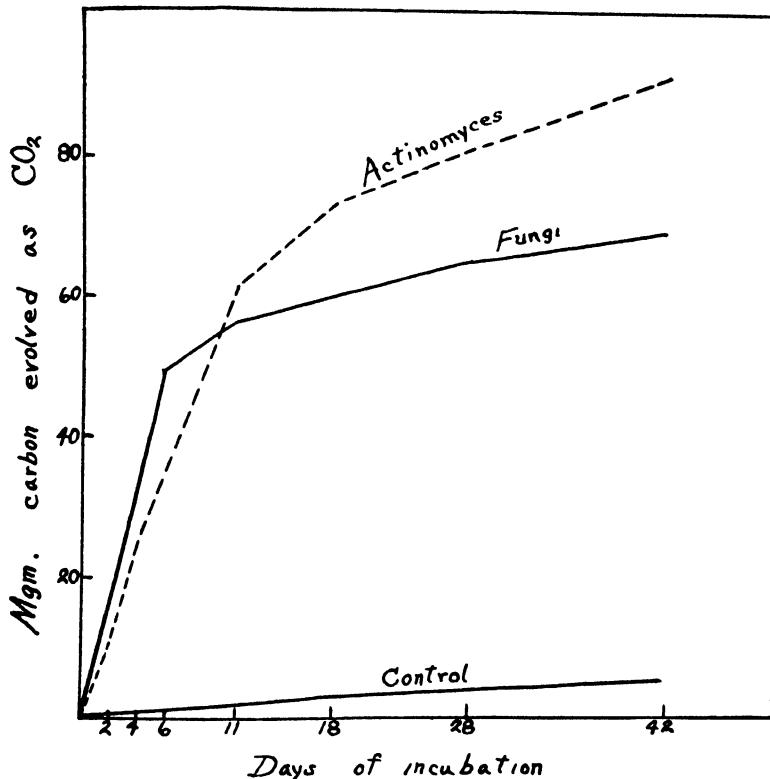
FIG. 1. THE RATE OF DECOMPOSITION OF MANNAN IN SAND MEDIUM BY PURE CULTURES OF
FUNGI AND ACTINOMYCES

In all the previous experiments only the total quantities of hemicellulose decomposed over a definite period of time were considered. In order to ascertain also the relative speed of decomposition of the various hemicelluloses, the carbon dioxide evolved in the process of decomposition was measured at short intervals over an extended period of time. The sand medium was utilized for this purpose. Carbon dioxide-free air was drawn through the cultures to displace the carbon dioxide in the flasks and this gas was absorbed in $N/6$ barium hydroxide solution. The excess barium hydroxide was titrated back with standard oxalic acid solution, at frequent intervals.

Table 16 shows the accumulative evolution of carbon dioxide, reported as milligrams of carbon, by the various fungi from mannan in sand culture. About 170 mgm. of carbon were added in the form of mannan; a little over one-third of this amount was liberated as CO_2 in 42 days. Since the fungi used represent several distinct types, it would appear that the mannan is readily decomposed by fungi in general and at about the same rate. Decom-
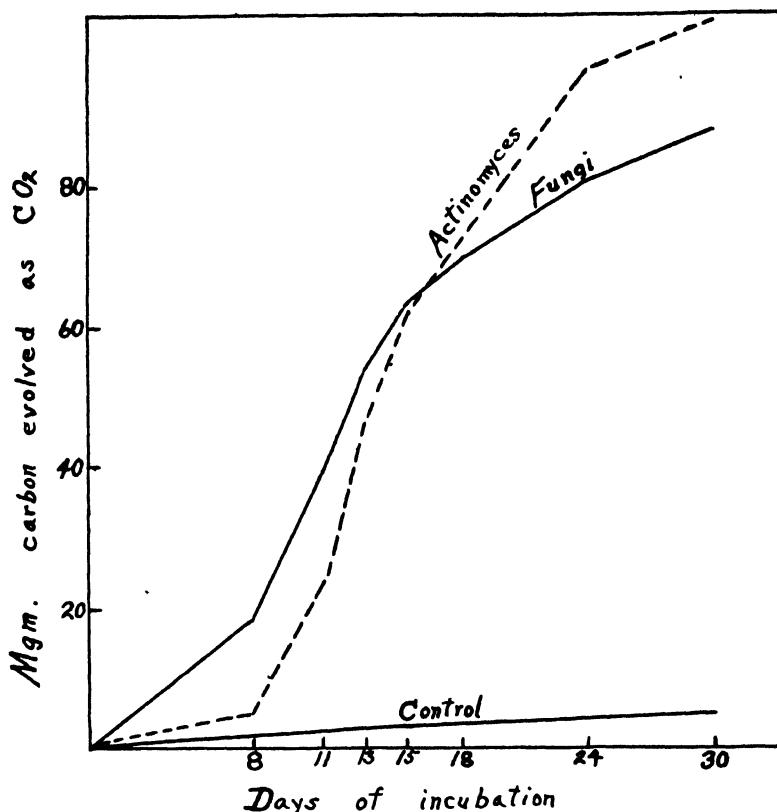
FIG. 2. THE RATE OF DECOMPOSITION OF MANNAN IN SALEP IN SAND MEDIUM BY PURE CULTURES OF FUNGI AND ACTINOMYCES

position was very rapid during the first 11 days; after that period, the rate of decomposition gradually decreased.

The liberation of carbon dioxide from mannan by actinomyces grown in sand medium is shown in table 17. A marked similarity in the total amounts of carbon evolved is again noted. The actinomyces were at first slower in bringing about the decomposition of the mannan than were the fungi, but they became more active later, finally resulting in the liberation of larger amounts of carbon dioxide. All the actinomyces liberated over one-half of the total carbon present originally in the mannan as carbon dioxide. Since the fungi

produce a more extensive mycelium than do the actinomyces, and use, therefore, more carbon for that purpose, the actual amount of mannan decomposed was probably the same, if not more, in the case of the fungi. This was brought out in tables 1–4.

These results show that, although the method of determination of carbon dioxide evolution as an index for measuring the rapidity of decomposition of plant material is very satisfactory, it cannot be used for comparing the activities of different groups of organisms which differ in their metabolism. This
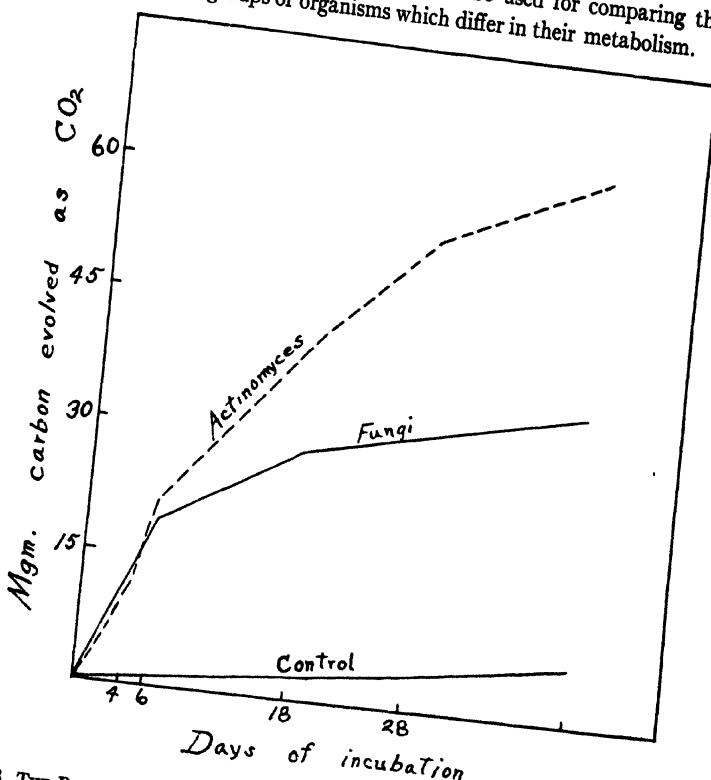
FIG. 3. THE RATE OF DECOMPOSITION OF XYLAN IN SAND MEDIUM BY PURE CULTURES OF FUNGI AND ACTINOMYCES

is true in the study of decomposition of more or less definite chemical complexes, but even more so in the study of unmodified plant materials. The same amount of carbon dioxide given off by different organisms may actually be a result of the decomposition of different plant constituents or different amounts of the same constituent.

Following the same plan of experiment, the evolution of carbon dioxide in the decomposition of natural mannan in the salep root was studied. It is of course possible and even certain that not all the carbon dioxide evolved in

this experiment came from the decomposition of the hemicellulose in the salep, but the mannan content is so large, in proportion to the other constituents, and the microörganisms have shown their ability to decompose the mannan so actively, that one is safe in assuming that most of the carbon liberated came from the mannan. The results of this experiment are given in table 18. The fungi showed the most active decomposition of the mannan in the early stages
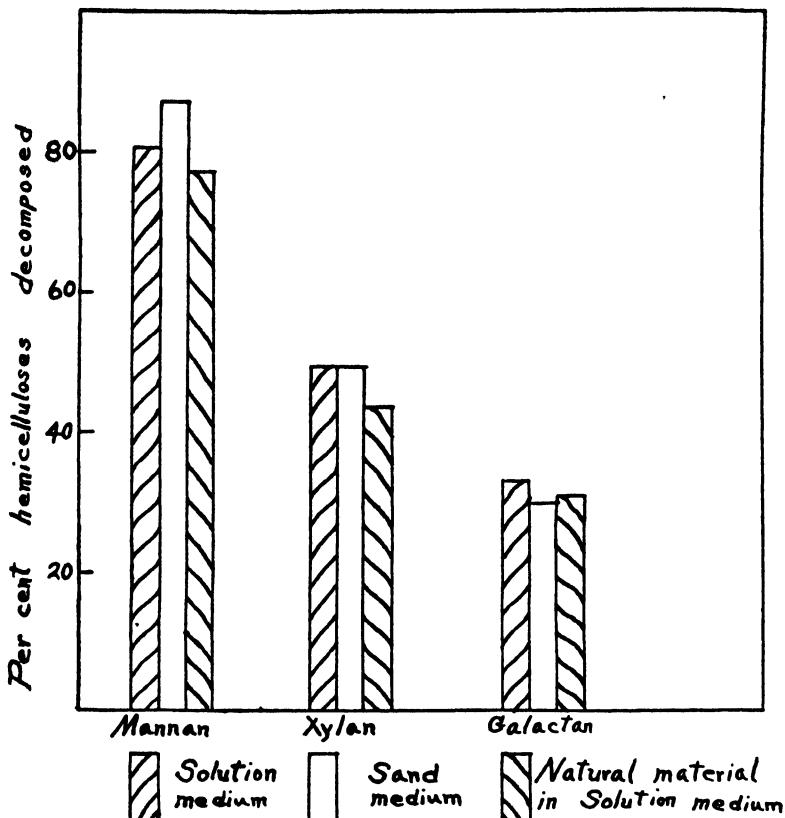
FIG. 4. THE TOTAL DECOMPOSITION OF THE VARIOUS HEMICELLULOSES BY FUNGI IN A PERIOD OF SIX WEEKS

of growth, but they did not slow down as much near the end of the incubation period as they did when the purified mannan was used as a source of energy.

The rate of decomposition of xylan from corn cobs, as shown by the evolution of carbon dioxide, was also studied. The plan of the experiment was identical to that used in the case of the mannan. Table 19 shows the amounts of carbon dioxide, reported as milligrams of carbon, evolved in the decomposition of xylan by pure cultures of fungi in a sand medium. Theoretically, about 207 mgm. of carbon were added to the medium in the form of xylan. The *Zygorhynchus* and *Rhizopus* were quite inactive in this experiment and gave off only

5.0 and 8.4 mgm. of carbon respectively, above the amount found in the un-inoculated control.   The remaining fungi liberated as carbon dioxide nearly 20 per cent of the carbon originally present in the xylan.

The liberation of carbon dioxide by the actinomyces from xylan in sand medium is given in table 20.   Here again, the rapidity of evolution of carbon dioxide by the actinomyces is more gradual than in the case of the fungi, but
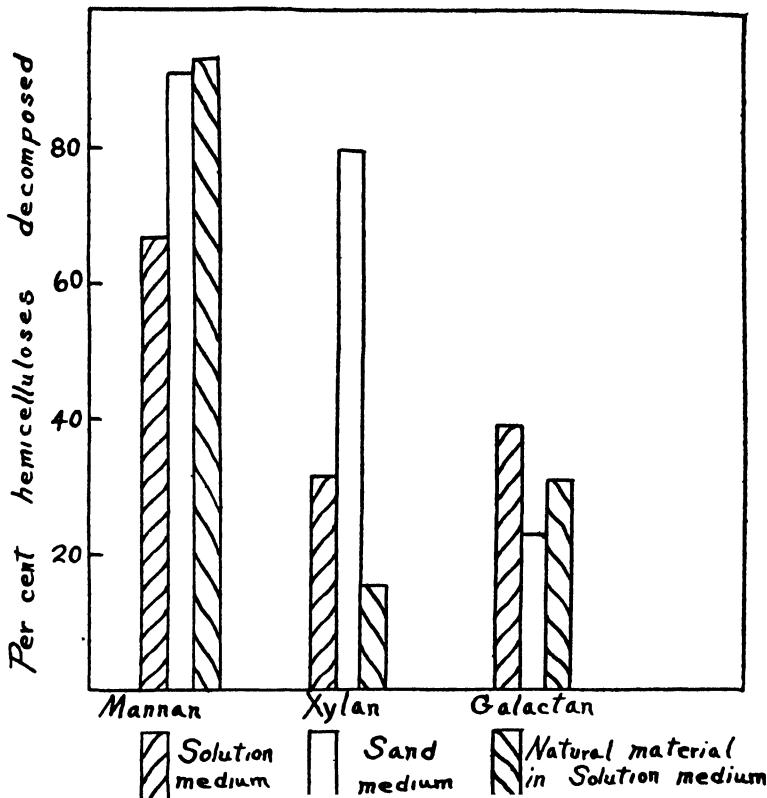
FIG. 5. THE RATE OF DECOMPOSITION OF THE VARIOUS HEMICELLULOSES BY ACTINOMYCES
IN A PERIOD OF SIX WEEKS

it is also more prolonged.   With one exception, over 25 per cent of the carbon in the xylan was liberated as carbon dioxide by the actinomyces.

Very little can be said at the present time concerning the mechanism of decomposition of the hemicelluloses by fungi and actinomyces.   A certain amount of organic acids is formed by some of the fungi.   The nature of these acids is not known, the amount varying with the organism, period of incubation, nature and composition of medium, etc.   In no case did the actinomyces produce any organic acids in the cultures.   This suggests definitely that the different organisms decompose the hemicelluloses in a different manner.

The average rate of decomposition and the total decomposition of the three "hemicelluloses," in a natural and purified state by fungi and actinomyces is brought out graphically in figures 1 to 5.

SUMMARY

A study has been made of the decomposition of several typical hemicelluloses, both in a purified form and in the natural plant material, by certain pure cultures of fungi and actinomyces.

The galactan was found to be more resistant to decomposition by microörganisms than were the mannan and xylan.

All the fungi tested were able to decompose the specific hemicelluloses; the Phycomycetes were as active in this process as the cellulose-decomposing fungi used in these experiments.

The actinomyces were found to be even more active in the decomposition of hemicelluloses than the fungi, especially under favorable conditions of culture.

When the rate of decomposition of hemicellulose by microörganisms was compared, measuring the evolution of carbon dioxide, the fungi were found to be more active in the beginning of the incubation period, but became considerable slower after decomposition has proceeded for about a week. The actinomyces were slow at the beginning of incubation, but maintained a more uniform rate of decomposition throughout a longer period of time.

The type of hemicellulose was found to be the most important determining factor in controlling the actual amount of decomposition by fungi. The actinomyces, however, were influenced not only by the type of hemicellulose but also by the environmental conditions at which decomposition was taking place.

Both fungi and actinomyces liberate considerable amounts of carbon dioxide in the process of destruction of hemicelluloses. The former also produces small amounts of organic acids in this process.

It appears that the process of preparation and purification of the hemicelluloses influences the nature and rate of their decomposition by microörganisms.

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ON THE DECOMPOSITION OF HEMICELLULOSES BY MICROÖR-GANISMS: III. DECOMPOSITION OF VARIOUS HEMICEL-LULOSES BY AEROBIC AND ANAEROBIC BACTERIA[1]

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Although it has been definitely established that various hemicelluloses are decomposed under both aerobic and anaerobic conditions, the literature on the decomposition of organic complexes by microörganisms contains but few references concerning the ability of specific aerobic and anerobic bacteria to decompose this group of carbohydrates. When compared with the interest aroused by the bacteria decomposing cellulose, the breakdown of hemicelluloses does not even occupy a second place. This is due to a number of factors; namely, the fact that the latter are not so abundant quantitatively and are not of so universal occurrence as the former; a better understanding of the chemistry of cellulose and the confused state of our knowledge of the chemistry of hemicelluloses; the fact that the bacteria decomposing the hemicelluloses are not so specific in their metabolism as the cellulose-decomposing bacteria; the greater abundance of bacteria capable of bringing about the decomposition of hemicelluloses.

Hoppe-Seyler demonstrated in 1889 (10) that xylans are decomposed by bacteria found in river mud. The ability of many bacteria to decompose pentosans was later established by a number of other investigators; the same was known to be true of the degradation of mannans (16, 17); however, the literature is very meager concerning the ability of specific bacteria to attack galactans, so that it came to be recognized that these compounds are very resistant to microbial action.

The rôle of anaerobic bacteria in the decomposition of hemicelluloses in the digestive tract of ruminants (11, 12, 20) and in the manure pile (21) has been known for a long time. It has been shown (19), for example, that the microorganisms in the large intestine of guinea pigs convert xylan into acetic and butyric acids, in the proportion of 9 parts of the former to 1 part of the latter. The feces of animals was found (20) to contain bacteria which produce acetic, propionic, and butyric acids, as well as traces of optically inactive lactic acid, from hemicelluloses. Anaerobic bacteria were found to be able to attack not

only pentosans but also methyl-pentosans (3). In the formation of silage by bacteria, a small amount of the pentosan was found (15) to be decomposed.

A number of aerobic bacteria were shown to be able to destroy hemicelluloses. This was true of the decomposition of the pentosan in green corn stover; *Bac. flavigena*, a cellulose-decomposing form, was found to be the most active organism. The specific decomposition of hemicelluloses by aerobic bacteria has been especially noted in the liquefaction of agar-agar, accompanied by hydrolysis; among the organisms capable of bringing about this process, one need only mention *Vibrio andoi* (1), *Bacillus nenckii* (4), *Bac. gelaticus* (8), *Microspira agar-liquefaciens* (9), *Bact. betae viscosum Panek*, and others (2, 16). Xylans were found to be decomposed by *Bac. mesentericus ruber* and *Bac. thermophilus grignoni* (7). In the infection of vegetables by *Bac. oleraceae* the hemicelluloses are known to be attacked. This is especially true of the pectins which are attacked by certain disease-producing bacteria, as in the case of the soft roots of carrots and other vegetables; the chemistry of the process is still imperfectly understood, although specific enzymes are known to be secreted by the organisms.

Mannans are attacked readily by *Bac. mesentericus vulgatus* (5) and by various soil bacteria (13, 16). Some of the bacteria produce tri-mannose from mannan (16); others produce laevudilin, a product of incomplete hydrolysis (13). Swartz (22) reported that the readiness with which salep (a source of mannan) disappears in the alimentary tract and is decomposed by fecal bacteria is similar to that of inulin. It is also interesting to note here that when uronic acids are decomposed by bacteria, the corresponding pentose may first be formed, as found by Salkowski and Neuberg (17) in the formation of *d*-xylose from glucuronic acid by bacteria.

It has been shown previously (24) that when plant residues are added to the soil, the hemicelluloses undergo rapid decomposition, not at a uniform rate, however. It has also been shown that numerous soil fungi and actinomyces are capable of decomposing large quantities of various hemicelluloses, liberating a part of the carbon as CO_2 and assimilating a part for the synthesis of microbial cell substance, as measured by the transformation of the nitrogen from an inorganic into an organic form. The galactans were found to be more resistant to decomposition than the mannans and xylans. The possibility has also been suggested (6) that certain hemicelluloses may be used as sources of energy by non-symbiotic nitrogen-fixing bacteria, without hydrolysis of the hemicellulose having to take place first by other bacteria, as found in the case of cellulose.

In order to study the decomposition of hemicelluloses by various typical soil bacteria, it was decided to isolate from the soil as many aerobic and anaerobic bacteria as possible; no attempt has been made, however, to make at the present time a detailed classification and identification of these organisms; the sole purpose was to test first the ability of these organisms to utilize these carbohydrates as sources of energy. Some of the cultures were saved for

further study, since they presented special interest, either because of the rapidity of hemicellulose decomposition that they brought about or because they formed special products of decomposition.

ISOLATION OF BACTERIA FROM SOIL

For the isolation of the bacteria, special media rich in hemicelluloses were employed. The cultures were isolated and tested first qualitatively, so as to determine which type of hemicellulose could supply the necessary energy for their life processes. Differences in specificity toward the various hemicelluloses were noted immediately. Some of the bacteria were able to decompose only one type of hemicellulose, others acted upon two different hemicelluloses, and still others were able to thrive on all the hemicelluloses studied. Table 1 gives a list of the bacteria isolated by the use of various carbohydrates as sources of energy and their ability to decompose the hemicellulose preparations as well as other carbohydrates. Attention should be called here to the fact that the mannan and galactan preparations were not pure hemicelluloses. The mere growth of an organism in the media containing these preparations is no proof at all that the organism is capable of using these as sources of energy. This is true especially of the galactan, as shown later in the quantitative studies.

DECOMPOSITION OF HEMICELLULOSES BY AEROBIC BACTERIA

For a more quantitative study of the decomposition of hemicellulose by bacteria, mannan prepared by the procedure outlined previously was at first employed. The tests were conducted in both sand and solution media. Each 100 cc. of solution or 100 gm. of sand contained the following ingredients:

	gm.
$(NH_4)_2HPO_4$	0 05
K_2HPO_4	0 05
$MgSO_4$	0.01
NaCl	0 01
$CaCl_2$	0 01
$FeSO_4$	0 001
Mannan preparation	(· 50

The sand cultures were kept at 20 per cent moisture so as to allow optimum aerobic conditions for the activities of the bacteria. Inoculations were made after the materials had been sterilized; the cultures were then incubated at 27 to 28°C. for 6 weeks. At the end of the incubation period, the contents of the flasks were analyzed for total hemicellulose and for ammonia. Hydrolysis of the hemicellulose was effected by autoclaving with 2 per cent HCl under 15 pounds pressure for one-half hour. This method is possible only when there are no other polysaccharides present which would give reducing sugars upon hydrolysis. Ammonia determinations were made by distillation with magnesium oxide.

TABLE 1

List of aerobic and anaerobic bacteria isolated from soil on media containing various carbohydrates and their ability to decompose these carbohydrates

NUMBER OF CULTURE	AEROBISM OR ANAEROBISM	CARBOHYDRATE USED FOR ISOLATION	ABILITY TO UTILIZE CARBOHYDRATES				
			Glucose	Starch	Mannan	Galactan	Xylan
1	Aerobic	Pentosan	+	+	−	++	+*
2	Aerobic	Pentosan	+	+	+	++	−
3	Aerobic	Mannan	+	+	+	++	+
4	Aerobic	Mannan	+	+	++	+	+
5	Anaerobic	Mannan	+	+	++	+	−
6	Aerobic	Mannan	+	+	−	+	−
7	Aerobic	Galactan	+	−	−	+	−
8	Aerobic	Galactan	+	+	++	+	++
9	Aerobic	Galactan	+	+	+	++	+
10	Aerobic	Galactan	+	−	+	++	−
11	Aerobic	Galactan	+	+	+	++	−
12	Aerobic	Glucose	....	....	....	. ..	....
13	Aerobic	Pentosan	+	−	−	+	−
14	Aerobic	Mannan	+	−	−	+	−
15	Aerobic	Galactan	....	....	.. .	....	....
16	Aerobic	Glucose	....	....	....	. ..	....
17	Aerobic	Glucose	+	+	−	+	−
18	Aerobic	Pentosan	....	....	....	....	. ..
19	Aerobic	Pentosan	. ..	. ..	....	....	....
20	Aerobic	Pentosan	+	+	+	++	−
21	Aerobic	Mannan	....	....	....	....	....
22	Aerobic	Mannan	+	+	+	++	−
23	Aerobic	Galactan	. ..	. .	....	....	....
24	Aerobic	Galactan	....	....	....	....	. .
25	Aerobic	Mannan	. .	....	....	....	....
27	Aerobic	Galactan	+	+	+	++	−
29	Aerobic	Pentosan	+	+	+	++	++
30	Aerobic	Pentosan	+	+	+	++	−
32	Aerobic	Xylan	....	....	....	. .	....
34	Aerobic	Mannan	+	+	+	++	+
36	Aerobic	Galactan	+	+	+	++	+
37	Aerobic	Pentosan	+	+	+	+	+
38	Aerobic	Pentosan	+	+	++	+	−
39	Aerobic	Xylan	....	....	....	....	....
41	Aerobic	Mannan	+	+	+	+	+
42	Aerobic	Mannan	....	....	....	....	....
44	Aerobic	Galactan	+	+	++	+	−
45	Aerobic	Galactan	+	+	+	+	−
46	Aerobic	Xylan	. ..	....	....	....	....
55	Aerobic	Lignin	+	−	+	++	+
56	Aerobic	Lignin	+	−	++	++	+

* − = no growth.

+ = positive growth.

++ = very good growth.

TABLE 1—*Concluded*

NUMBER OF CULTURE	AEROBISM OR ANAEROBISM	CARBOHYDRATE USED FOR ISOLATION	ABILITY TO UTILIZE CARBOHYDRATE				
			Glucose	Starch	Mannan	Galactan	Xylan
57	Aerobic	Lignin	+	+	++	+	+
58	Aerobic	Lignin	−	−	+	+	−
59	Aerobic	Lignin	+	−	+	+	+
60	Aerobic	Starch	+	+	+	++	+
61	Aerobic	Starch	+	+	++	+	−
62	Aerobic	Starch	+	+	+	++	+
63	Aerobic	Starch	+	+	++	+	−
64	Aerobic	Starch	+	+	+	+	++
65	Aerobic	Starch	+	+	+	++	−
66	Aerobic	Starch	+	+	+	+	−
67	Aerobic	Starch	+	−	+	++	−
68	Aerobic	Mannan	−	−	+	+	+
69	Aerobic	Mannan	+	+	+	++	+
70	Anaerobic	Mannan	−	−	++	+	−
71	Aerobic	Mannan	−	+	+	++	+
72	Aerobic	Mannan	+	−	++	+	+
73	Aerobic	Mannan	+	+	+	+	+
75	Aerobic	Galactan			.		..
77	Aerobic	Galactan	+	+	+	+	+
79	Aerobic	Galactan					... .
80	Aerobic	Xylan					.
81	Aerobic	Xylan					..
82	Aerobic	Xylan					. .
83	Aerobic	Xylan					..
84	Aerobic	Xylan					. .
85	Aerobic	Xylan					
86	Anaerobic	Galactan	+	+	+g†	+g	?
87	Anaerobic	Mannan	+	−	+g	+g	−
88	Anaerobic	Mannan	+	+	+	+	−
89	Anaerobic	Mannan	+	+	+g	+	?
90	Anaerobic	Mannan	+	−	−	+	?
91	Anaerobic	Mannan	+	+	+g	+g	−
92	Anaerobic	Xylan	+	+g	+	+	−
93	Anaerobic	Xylan	+	+	+	+	−
94	Anaerobic	Xylan	+	+	+	+	−
95	Anaerobic	Xylan	+	−	+g	+g	−
96	Anaerobic	Xylan	+	−	+g	+g	−
97	Anaerobic	Xylan	+	−	+g	+g	−

† g = gas evolution under anaerobic conditions.

The decomposition of mannan in sand media by pure cultures of bacteria is shown in table 2. All of the bacteria tested decomposed the mannan very actively, in some cases nearly 95 per cent of the hemicellulose disappearing within a period of 6 weeks. Even the least active organism, namely, Bacterium 83 decomposed nearly half of the mannan present, in this period of time. On the average, 33.5 parts of mannan were decomposed for each unit of nitro-

TABLE 2

The decomposition of mannan in sand medium by pure cultures of soil bacteria

INOCULUM	MANNAN		AMMONIA-NITROGEN		PARTS OF MANNAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
	Found	Decomposed	Found	Assimilated	
	mgm.	mgm.	mgm.	mgm.	
Control.....	305.5	0	9.6	0	....
Bacterium 4..............	74.5	231.0	5.0	4 6	50 2
Bacterium 8..................	74.5	231 0	0 1	9.5	24.3
Bacterium 55.... . :	12.4	293.1	5.1	4.5	65.1
Bacterium 56.....	17.8	287.7	2 1	7.5	38.4
Bacterium 62....	52 9	252.6	3.4	6.2	40.7
Bacterium 64	116.1	189.4	2 0	7.6	24.9
Bacterium 2....	52.9	242 6	2.5	7.1	34.2
Bacterium 58....	15.1	290.4	1.0	8.6	33.8
Bacterium 69	15.1	290 4	2.9	6.7	43.3
Bacterium 5...	26.1	279.4	1 9	7.7	36.3
Bacterium 9..... ,.	30 6	274 9	3.0	6.6	41.6
Bacterium 10...	46 4	259 1	1.0	8 6	30 1
Bacterium 11....	44 1	261 4	0 4	9 2	28 4
Bacterium 20	26.1	279 4	2.7	6 9	40.5
Bacterium 22	23.9	281 6	1 9	7.9	35.7
Bacterium 27....	55 8	249.7	1.0	8 6	29 0
Bacterium 28.....	37 4	268 1	2 0	7.6	35.3
Bacterium 30....	26 1	279 4	3 0	6 6	42.3
Bacterium 34...	73 8	231 7	0 7	8 9	26 0
Bacterium 36... .. .	44 1	261 4	0 1	9 5	27.5
Bacterium 38	41 9	263 6	0 7	8 9	29.6
Bacterium 41...	59 9	245 6	2.0	7 6	32 3
Bacterium 43...	41 9	263 6	0.2	9 4	28 0
Bacterium 44...	28.4	277.1	0 7	8 9	31.1
Bacterium 45....	26 1	279 4	1 1	8 5	32.9
Bacterium 53... . . .	28 4	277 1	2.2	7 4	37.4
Bacterium 57...	44 1	261.4	1 0	8 6	30 4
Bacterium 59....	28 4	277 1	0 6	9 0	30.8
Bacterium 61....	28 4	277 1	0.7	8 9	31.1
Bacterium 63...	41 9	263 6	0 4	9 2	28.7
Bacterium 65...	32 9	272 6	0 9	8 7	31 3
Bacterium 66...	32 9	272 6	0 5	9 1	30 0
Bacterium 67	30 6	274 9	0 6	9 0	30 6
Bacterium 68...	37 4	268 1	1.1	8 5	31 5
Bacterium 83...	157.1	148 4	2 4	7.2	20.6
Bacterium 71	113 4	192 1	0 5	9 1	21 1
Bacterium 72	106 2	199 3	0 7	8.9	22 4
Bacterium 73	37.4	268 1	4 0	5 6	47.9
Bacterium 75	48 6	256 9	2 1	7 5	34.3
Bacterium 77....	50 9	254 6	1 4	8 2	31 1
Bacterium 79...... ....:.....	53 6	251 9	2.5	7.1	35 5
Bacterium 82.......................	30 6	274 9	1.2	8.4	32.7

TABLE 3

The decomposition of mannan in solution medium by pure culture of soil bacteria

BACTERIUM	pH	MANNAN		AMMONIA-NITROGEN		PARTS OF MANNAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
		Found	Decomposed	Found	Assimilated	
		mgm	*mgm*	*mgm*	*mgm*	
Control	5 4	382 5	0	9 6	0	.
Bacterium 4	5 2	270 5	112 0	6 2	3 4	32 9
Bacterium 8	5 2	334 8	47 7	7 6	2 0	23 9
Bacterium 55.	5 2	265 5	117 0	5 2	4 4	26 6
Bacterium 56	5 2	338 0	44 5	8 0	1 6	28 3
Bacterium 62	5 2	280 8	101 7	5 0	4 6	22 1
Bacterium 64	5 0	213 8	168 7	1 6	8 0	21 1
Bacterium 2	5 2	310 5	62 0	7 0	2 6	23 9
Bacterium 58	5 2	334 8	47 7	7 6	2 0	23 9
Bacterium 69	5 1	340 7	31 8	8 1	1 5	21 2
Bacterium 5	5 2	326 7	55 8	8 4	1 2	46 5
Bacterium 9	5 0	156 6	225 9	1 6	8 0	28 2
Bacterium 10	5 4	352 4	30 1	7 4	2 2	13 7
Bacterium 11	5 4	310 5	72 0	3 0	6 6	10 9
Bacterium 20	5 4	349 2	33 3	6 4	3 2	10 4
Bacterium 22	5 2	286 7	95 8	5 8	3 8	24 7
Bacterium 27	5 2	326 7	55 8	7 8	1 8	31 0
Bacterium 28	5 2	318 6	63 9	5 6	4 0	16 0
Bacterium 30	5 2	318 6	63 9	8 4	1 2	53 3
Bacterium 38	5 2	297 0	85 5	7 2	2 4	35 5
Bacterium 41	5 2	310 5	72 0	5 2	4 4	16 4
Bacterium 44	5 2	305 1	77 4	6 8	2 8	27 6
Bacterium 45	4 8	162 0	220 5	0 4	9 2	23 9
Bacterium 53	5 2	46 4	336 1	2 4	7 2	46 7
Bacterium 57	5 2	329 4	53 1	6 2	3 4	15 6
Bacterium 59	5 2	307 4	75 1	4 8	4 8	15 6
Bacterum 61	5 2	268 2	114 3	4 8	4 8	23 8
Bacterium 63	5 2	351 5	31 0	7 0	2 8	11 1
Bacterium 65	5 0	118 4	264 1	1 2	8 4	31 4
Bacterium 66	4 8	96 8	285 7	0 0	9 6	29 8
Bacterium 67	4 6	186 3	196 2	0 2	9 4	20 9
Bacterium 68	5 2	284 9	97 6	4 8	4 8	20 3
Bacterium 83	4 8	92 3	290 2	0 2	9 4	30 9
Bacterium 71	5 2	360 0	22 5	7 4	2 2	10 2
Bacterium 72	5 2	297 0	85 5	4 4	5 2	16 4
Bacterium 73	5 2	346 1	36 4	7 4	2 2	16 5
Bacterium 75	5 2	348 3	34 2	6 8	2 8	12 2
Bacterium 77	5 2	273 6	108 9	6 8	2 8	38 9
Bacterium 79	5 2	348 3	34 2	7 2	2 4	14 3
Bacterium 82	5 2	297 0	85 5	5 8	3 8	22 5

gen assimilated by the bacteria. Considerable variation was, of course, noted, but because of the large number of cultures used this figure is very significant in respect to the nitrogen utilization by microörganisms decomposing the hemicelluloses. The average amount of mannan decomposed by the 42 different cultures of bacteria was 258.3 mgm., constituting 84.7 per cent of the hemicellulose added. It is to be expected, therefore, that since the mannan is so readily decomposed by bacteria in sand media, it will also be decomposed to a considerable extent in the soil by these microörganisms.

The decomposition of mannan in solution medium by pure cultures of bacteria is shown in table 3. A rather wide variation of the amounts of mannan decomposed is here noted. On the average, 103.5 mgm. of mannan, out of the original 382.5 mgm., were decomposed. For every part of nitrogen assimilated

TABLE 4

The decomposition of mannan in salep roots by pure cultures of soil aerobic bacteria

ORGANISM	pH	MANNAN		AMMONIA NITROGEN		PARTS OF MANNAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
		Found	Decom posed	Found	Assimi lated	
		mgm	*mgm*	*mgm*	*mgm*	
Control	7 0	660 4	0	62 0	0	
Bacterium 4	7 0	545 4	115 0	54 8	7 2	16 0
Bacterium 8	6 8	383 3	278 1	55 2	6 8	40 9
Bacterium 55	6 4	362 9	297 5	55 6	6 4	46 5
Bacterium 62	6 5	105 3	555 1	45 6	16 4	33 8
Bacterium 64	6 4	317 0	343 4	50 4	11 6	29 6
Bacterium 2	6 9	542 7	117 7	52 8	9 2	12 8
Bacterium 58	6 6	80 6	579 9	48 8	13 2	43 9
Bacterium 36	7 0	346 7	313 7	58 0	4 0	78 4
Bacterium 10	7 0	620 5	39 9	60 0	2 0	20 0
Bacterium 11	6 6	375 3	285 1	48 8	13 2	21 6
Bacterium 20	6 9	530 3	130 1	52 4	9 6	13 6
Bacterium 28	6 8	382 3	278 1	54 0	8 0	34 8

by the bacteria, 14.1 units of mannan were decomposed. The small amount of decomposition and the large amount of nitrogen consumed may be due to the fact that these organisms are essentially aerobic and conditions in the solution cultures were not so favorable for their development and for their physiological activities as they were in the sand cultures.

These results were obtained by the use of purified mannan preparations. In order to determine whether or not the extraction and preparation of the mannan had any influence upon its decomposition, the mannan in the natural state (salep root) was subjected to the activities of the bacteria showing the greatest amount of decomposition on the purified product. The results of this experiment are given in table 4.

The various aerobic bacteria destroyed varying amounts of mannan ranging

up to 87.8 per cent of the total mannan present. For every unit of nitrogen assimilated, the bacteria decomposed, on the average, 32.6 units of mannan.

TABLE 5

The decomposition of xylan in sand medium by pure cultures of soil aerobic bacteria

BACTERIUM	XYLAN		AMMONIA-NITROGEN		PARTS OF XYLAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
	Found	Decomposed	Found	Assimilated	
	mgm	*mgm*	*mgm*	*mgm*	
Control .	152 0	0	8 6	0	
Bacterium 8 .	88 2	63 8	6 3	2 3	27 7
Bacterium 24	92 7	59 3	5 9	2 7	22 0
Bacterium 28	83 7	68 3	4 3	4 3	15 9
Bacterium 29 .	38 7	113 3	1 9	6 7	16 9
Bacterium 1 .	65 7	86 3	2 2	6 4	13 5
Bacterium 9.	107 1	44 9	5 3	3 3	13 6
Bacterium 41	110 7	41 3	6 6	2 0	20 7
Bacterium 43	88 6	63 4	2 8	5 6	11 3
Bacterium 53	48 4	107 2	3 2	5 4	19 9
Bacterium 55	99 4	52 6	7 0	1 6	32 9
Bacterium 59	113 4	38 6	6 6	2 0	19 3
Bacterium 62	96 7	55 3	5 6	3 0	18 4
Bacterium 68	113 4	38 6	5 9	2 7	14 3

TABLE 6

Decomposition of xylan in solution media by pure cultures of soil aerobic bacteria

BACTERIUM	pH	XYLAN		AMMONIA-NITROGEN		PARTS OF XYL N DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
		Found	Decomposed	Found	Assimilated	
		mgm	*mgm*	*mgm*	*mgm*	
Control	6 2	287 3	0	8 6	0	
Bacterium 4	6 2	271 8	15 5	8 2	0 4	38 8
Bacterium 8	6 2	205 8	81 5	3 6	5 0	16 3
Bacterium 24	6 2	156 2	131 1	3 6	5 0	26 2
Bacterium 28	6 2	238 2	49 1	7 6	1 0	49 1
Bacterium 29	6 2	138 6	148 7	3 6	5 0	29 7
Bacterium 64	6 2	138 6	148 7	4 6	4 0	37 2
Bacterium 1	6 2	267 6	19 7	8 2	0 4	49 3
Bacterium 3	6 2	258 6	26 7	8 2	0 4	66 8
Bacterium 34	6 2	275 4	11 9	.8 2	0 4	24 8
Bacterium 36	6 2	278 4	8 9	8 0	0 6	14 9
Bacterium 43	5 8	157 8	129 5	2 8	5 8	22 3
Bacterium 53	6 2	241 8	45 5	8 4	0 2	

It is to be remembered that the nitrogen utilization values in the case where the natural materials were used are not as exact and reliable as they are in the

experiments where the purified products were employed, because in the former case the organisms may have attacked other substances, while the nitrogen in the salep root itself may have been preferred to the nitrogen added.

The experiments with the xylan preparation followed the same plan as outlined for the mannan. The flasks were inoculated with organisms which had

TABLE 7

The decomposition of xylan in corn cobs in solution media by pure cultures of soil aerobic bacteria

ORGANISM	XYLAN		AMMONIA-NITROGEN		PARTS OF XYLAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
	Found	Decom posed	Found	Assimi lated	
	mgm	*mgm*	*mgm*	*mgm*	
Control	405 5	0	30 0	0	
Bacterium 43	368 8	36 7	28 0	2 0	18 4
Bacterium 64	372 1	33 4	29 2	0 8	41 8
Bacterium 8	241 9	163 6	26 8	3 2	51 1
Bacterium 28	250 6	154 9	27 6	2 4	64 5
Bacterium 53	271 6	133 9	24 4	5 6	23 9
Bacterium 1	167 9	237 6	24 0	6 0	39 6

TABLE 8

Decomposition of galactan in solution media by pure cultures of soil aerobic bacteria

ORGANISM	GALACTAN		AMMONIA NITROGEN	
	Found	Decomposed	Found	Assimilated
	mgm	*mgm*	*mgm*	*mgm*
Control	306 9	0	9 3	0
Bacterium 1	144 9	162 0	8 3	1 0
Bacterium 2	176 4	130 5	9 0	0 3
Bacterium 4	173 7	133 2	9 0	0 3
Bacterium 9	157 5	148 4	8 7	0 6
Bacterium 11	229 5	77 4	8 3	1 0
Bacterium 17	269 1	37 8	9 0	0 3
Bacterium 20	252 0	54 9	9 0	0 3
Bacterium 22	261 9	45 0	8 7	0 6
Bacterium 41	269 1	37 8	8 0	1 3
Bacterium 27	229 5	77 4	8 7	0 6
Bacterium 34	265 5	41 4	8 0	1 3

shown, in previous trials, that they are capable of decomposing the xylan. The incubation period was 6 weeks at a temperature of 27° to 28°C. The methods of analysis were the same as those employed in the experiment with mannan.

The decomposition of the xylan in sand media by pure cultures of aerobic bacteria is reported in table 5. In only two cases was more than two-thirds of

the xylan destroyed. The average amount of xylan decomposed was 42.1 per cent. In this study, 18.9 parts of the xylan were utilized per unit of nitrogen assimilated. There was a close agreement in these values for the various bacteria.

The decomposition of the xylan in solution media by the aerobic bacteria is shown in table 6. On the average, 23.7 per cent of the xylan was decomposed, but in no case did the amount destroyed reach much more than 50 per cent of the total material originally present. In solution media the bacteria used 1 part of nitrogen for every 31.3 parts of xylan decomposed.

The ability of the bacteria to decompose xylan in the natural state was also determined. Since the xylan preparation had been obtained from corn cobs, this source of material was employed to study the decomposition of xylan as

TABLE 9

Decomposition of galactan in Irish moss in solution media by pure cultures of soil aerobic bacteria

ORGANISM	GALACTAN		AMMONIA-NITROGEN		PARTS OF GALACTAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
	Found	Decom posed	Found	Assimi-lated	
	mgm	*mgm*	*mgm*	*mgm*	
Control	382 3	0	50 0	0	
Bacterium 2	263 0	119 3	46 0	4 0	29 8
Bacterium 9 .	321 8	60 5	46 0	4 0	15 1
Bacterium 11	321 8	60 5	45 2	4 8	12 6
Bacterium 17 .	291 1	91 2	46 0	4 0	22 8
Bacterium 20	287 1	95 2	46 4	3 6	26 4
Bacterium 22	344 0	38 3			
Bacterium 41	360 2	22 1			
Bacterium 27	287 1	95 2	48 0	2 0	47 6
Bacterium 34 .	271 6	110 7	43 2	6 8	16 3

it exists in nature   It should be remembered that xylan makes up only about 30 per cent of the total constituents of the corn cobs. Nevertheless, it was believed that the study of the activities of xylan-decomposing organisms upon the ground corn cobs would aid in the formulation of a clearer conception of the utilization of xylan by these bacteria. These studies were carried out in solution media and the results are given in table 7.

The bacteria decomposed, on the average, 31.2 per cent of the xylan present. This is about 8 per cent higher than the decomposition of the purified xylan under the same conditions. Nearly 40 parts of xylan were decomposed per unit of nitrogen assimilated.

The experiments dealing with the decomposition of galactan by various bacteria were carried out in exactly the same manner as those reported for the mannan and xylan. The galactan was found to be more resistant to decom-

position by microorganisms than were the other two hemicelluloses. Variation among bacteria in their ability to decompose the galactan was more marked than in the case of the other hemicelluloses. The results of the decomposition of the galactan in solution media by pure cultures of aerobic bacteria are given in table 8. In four cases, about half of the galactan was decomposed.

TABLE 10

Decomposition of mannan in salep roots by pure cultures of anaerobic bacteria

ORGANISM	pH	MANNAN		NH₃ N		PARTS OF MANNAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
		Found	Decom posed	Found	Assimi lated	
		mgm	*mgm*	*mgm*	*mgm*	
Control	6 6	770 0	0	114 4	0	
Bacterium 5	5 8	411 5	358 5	104 4	10 0	35 9
Bacterium 70	6 2	171 7	598 3	108 0	6 4	93 5
Bacterium 86	6 2	688 0	82 0	109 6	4 8	17 1
Bacterium 87	4 8	155 5	614 5	106 0	8 4	73 2
Bacterium 88	4 8	238 7	531 3	106 4	8 0	66 4
Bacterium 89	6 3	757 1	12 9	111 6	2 8	
Bacterium 90	4 8	128 5	641 5	107 2	7 2	89 1
Bacterium 91	5 4	429 8	340 2	112 8	1 6	21 3
Bacterium 92	5 2	341 3	428 7	108 4	6 0	71 5
Bacterium 93	6 2	526 0	244 0	102 4	12 0	20 3
Bacterium 94	5 6	388 8	381 2	110 4	4 0	95 3
Bacterium 95	5 4	411 5	358 5	107 6	6 8	52 7
Bacterium 96	5 2	429 8	340 2	109 6	4 8	70 9

TABLE 11

Composition of the gases produced by Bacterium 70 in the decomposition of mannan in salep roots

CONSTITUENT	(a)*	(b)	(c)
	per cent	*per cent*	*per cent*
Carbon dioxide (CO₂)	0 2	4 6	8 0
Oxygen (O₂)	3 9	2 2	2 0
Hydrogen (H₂)	2 7	0 0	0 0
Methane (CH₄), carbon monoxide, (CO), nitro-gen (N₂), etc	93 2	93 2	90 0

* (a) = first gas collection, 2,225 cc , (b) = second, 2,350 cc , (c) third, 2,500 cc

The remaining cultures decomposed a much smaller amount of this carbohydrate, so that, on the average, only 23.2 per cent of the galactan disappeared, as a result of bacterial action. It is interesting to note that the amounts of inorganic nitrogen used by the bacteria in the decomposition of the galactan were very small, possibly because of the utilization of some of the nitrogen found in the galactan preparation.

In order to compare the influence of treatment in the process of preparation of the galactan upon its decomposition by bacteria, the natural product, namely Irish moss, was employed as a source of energy for these bacteria. The results of these studies, shown in table 9, are similar to those obtained with the purified preparation. The resistance of the galactan to decomposition by various microörganisms is further brought out in this experiment. The bac-

TABLE 12

Composition of the gas produced by Bacterium 5 in the decomposition of the mannan in salep roots

CONSTITUENT	PER CENT OF TOTAL
Carbon dioxide (CO_2)	20 4
Oxygen (O_2)	0 4
Hydrogen (H_2)	45 4
Methane, carbon monoxide, nitrogen, etc	33 8

TABLE 13

Decomposition of galactan in Irish moss by pure cultures of anaerobic bacteria

ORGANISM	GALACTAN		NH₃ N		PARTS OF GALACTAN DECOMPOSED PER UNIT OF NITROGEN ASSIMILATED
	Found	Decom posed	Found	Assimi lated	
	mgm	*mgm*	*mgm*	*mgm*	
Control	531 4	0	112 8	0	
Bacterium 5	447 1	84 3	109 2	3 6	23 5
Bacterium 70 .	453 6	77 8	108 8	4 0	19 5
Bacterium 86		No growth			
Bacterium 87	459 0	72 4	109 6	3 2	22 6
Bacterium 88	435 2	96 2	108 0	4 8	20 0
Bacterium 89	465 5	65 9	109 6	3 2	20 6
Bacterium 90	441 7	89 7	107 6	5 2	17 3
Bacterium 92		No growth			
Bacterium 93	459 0	72 4	105 2	7 6	9 5
Bacterium 94	441 7	89 7	111 2	1 6	56 2
Bacterium 95		No growth			
Bacterium 96		No growth			
Bacterium 97 .		No growth			

teria were able to decompose only about 20.0 per cent of the galactan in the Irish moss. For every unit of nitrogen assimilated about 20 parts of galactan were decomposed.

DECOMPOSITION OF HEMICELLULOSES BY ANAEROBIC BACTERIA

In addition to the aerobic bacteria, a number of anaerobes were tested for their hemicellulose decomposing capacity. The anaerobic organisms were obtained from the soil by the use of media containing the various hemicelluloses

as sources of energy. They were allowed to develop in an atmosphere free from atmospheric oxygen Practically all of these bacteria were gas-formers when grown in solutions containing the specific hemicelluloses. Microscopic examination showed that they were all spore-forming rods of various sizes.

It was very difficult to grow these organisms on the purified hemicellulose preparations. In order to avoid great variation in the results, the bacteria were grown on the natural materials rich in hemicelluloses in liquid media.

TABLE 14

Evolution of carbon dioxide from mannan in sand medium by pure cultures of bacteria

CO_2 as mgm C

ORGANISM	DAYS OF INCUBATION						
	2	4	6	11	18	28	42
Control	0 1	0 3	0 7	2 0	3 5	4 0	5 2
Bacterium 2	1 7	18 0	36 7	48 9	55 4	61 0	72 2
Bacterium 4	3 0	3 1	6 2	16 6	30 2	42 3	55 2
Bacterium 8	8 3	23 0	36 6	44 6	53 7	65 7	81 7
Bacterium 55	16 6	25 8	42 7	54 5	68 1	81 1	98 3
Bacterium 56	8 4	26 5	35 5	43 6	49 8	52 0	64 7
Bacterium 58	9 2	27 6	34 8	41 5	49 0	56 3	67 6
Bacterium 62	12 6	27 2	39 6	54 6	73 0	83 3	88 2
Bacterium 64	18 4	35 8	47 8	55 1	61 4	68 7	80 7
Bacterium 69	16 4	29 8	38 6	44 3	51 2	65 6	83 1

TABLE 15

Evolution of carbon dioxide from mannan in salep roots by aerobic bacteria

CO_2 as mgm C

ORGANISM	DAYS OF INCUBATION						
	8	11	13	15	18	24	30
Bacterium 8	16 2	30 1	40 9	50 3	57 8	69 6	73 6
Bacterium 55	6 7	14 0	18 7	23 9	29 6	39 9	47 4
Bacterium 58	8 5	25 8	35 9	43 8	51 8	74 7	94 7
Bacterium 62	15 5	33 6	43 5	51 1	57 3	67 2	71 0

The decomposition of the mannan of salep root by pure cultures of anaerobic bacteria is shown in table 10. The media containing the necessary mineral nutrients were inoculated and incubated for 6 weeks. Only three of the organisms decomposed less than 45 per cent of the mannan present in the medium, whereas in one case as much as 83 per cent of the hemicellulose was decomposed. Large quantities of organic acids were produced in some cases. Considerable variation was observed in the nitrogen utilization, and, therefore, too much emphasis should not be placed upon the results. It is quite probable that, in addition to the mannan, other compounds of the salep root were de-

composed in some instances. This is shown by the fact that *Bacterium 89*, which decomposed the least amount of mannan, had produced a very heavy growth in the culture flask; this also accounts for the comparatively high consumption of the available nitrogen. A detailed study was made of the gases produced by *Bacterium 5* and *Bacterium 70*. The cultures were grown in liter flasks and the gas was collected over saturated solutions of NaCl. The gas produced by *Bacterium 70* was collected in three lots, as follows: (*a*) 2,225 cc.; (*b*) 2,350 cc.; and (*c*) 2,500 cc. The composition of these gases is given in table 11.

It is interesting to note that hydrogen was formed only at the beginning of the fermentation period. In no case was the sum of the methane, carbon monoxide, and nitrogen below 90 per cent of the total gas evolved. There was a steady increase in the carbon dioxide with a decrease in the oxygen.

TABLE 16

Evolution of carbon dioxide from xylan by pure cultures of bacteria

CO_2 as mgm C

ORGANISM	DAYS OF INCUBATION				
	4	6	18	28	42
Control..	0 3	0 8	2 5	3 8	5 1
Bacterium 1 .	1 7	2 5	17 5	35 7	43 4
Bacterium 3 ..	1 0	1 7	8 0	11 3	14 7
Bacterium 8	5 8	7 8	10 6	12 6	14 7
Bacterium 9 .	2 9	7 1	20 0	25 7	33 1
Bacterium 24	12 5	22 3	35 9	36 7	37 9
Bacterium 28 .	3 6	5 6	7 1	7 8	9 1
Bacterium 29	13 6	24 4	34 7	37 2	49 9
Bacterium 64 .	1 2	11 9	22 6	25 6	28 7

Bacterium 5 produced only 1,050 cc. of gas. The composition of this gas is given in table 12. The analysis of this gas shows that the processes of decomposition brought about by the two bacteria are widely different in character. Practically two-thirds of the gas produced by *Bacterium 5* consisted of carbon dioxide and hydrogen. Since almost half of the gas consists of hydrogen, this may be spoken of as essentially a hydrogen fermentation process. The amount of oxygen in this case is negligible. A detailed study of the morphological characters and physiological activities of this organism will be published later.

The decomposition of the galactan in the Irish moss by pure cultures of anaerobic soil bacteria is shown in table 13. The medium consisted of 1 per cent galactan in the form of Irish moss, added to a mineral solution. The decomposition process was allowed to proceed for 6 weeks. The pH of the solutions remained practically constant. This is no proof that organic acids were not produced in the decomposition of the galactan, but may be merely a

result of the buffering properties of the ammonium phosphate in the mineral solution and of the galactan itself. However, it indicated that only small amounts, if any, of organic acids were produced. In no case was more than 18 per cent of the galactan decomposed. This is in accord with the resistance which the galactan was shown to possess toward the action of microörganisms in the other experiments.
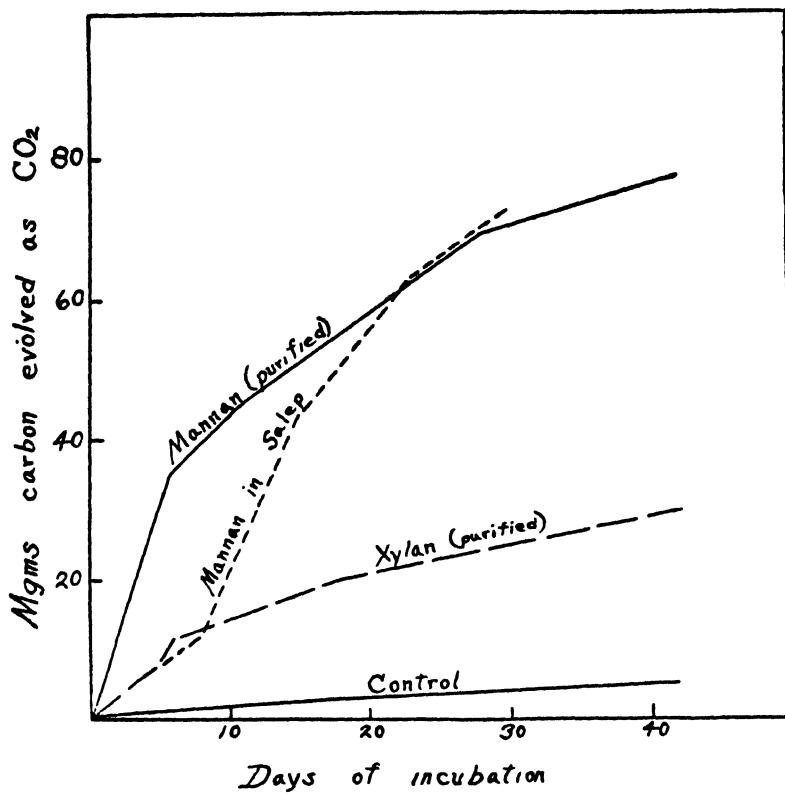
FIG. 1. COMPARISON OF THE RATE OF DECOMPOSITION OF CERTAIN HEMICELLULOSES BY AEROBIC BACTERIA

EVOLUTION OF CO_2 IN THE DECOMPOSITION OF HEMICELLULOSES

In order to ascertain the relative speed of decomposition of the various hemicelluloses by microörganisms, the carbon dioxide evolved was measured at short intervals over an extended period of time. The culture medium was identical with that outlined for the sand medium in the mannan experiment. Carbon dioxide-free air was drawn through the culture flasks to displace the carbon dioxide in the flasks and the latter was absorbed in $N/6$ solution of bar um hydroxide. The carbon dioxide produced was measured by frequent titrations of the excess barium hydroxide which served as a measure of the rate of decomposition.

Table 14 shows the carbon dioxide evolved by aerobic bacteria in the decomposition of mannan. The total amounts of carbon dioxide liberated, measured as carbon, ranged from 55.2 to 98.3 mgm. The various bacteria were found to exhibit different rates of decomposition. Some of them decomposed the mannan rapidly at the beginning and then more slowly; some acted slowly at first and more rapidly later, whereas others maintained practically an even rate of decomposition throughout the incubation period.
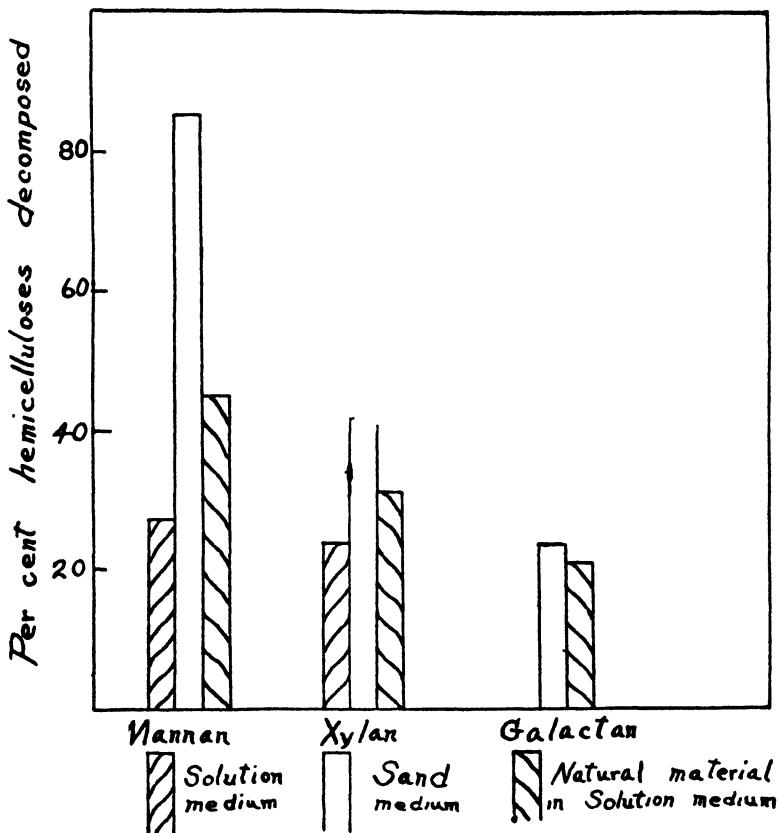
FIG. 2. EXTENT OF DECOMPOSITION OF VARIOUS HEMICELLULOSES BY AEROBIC
BACTERIA

Following the same plan of experiment, the evolution of carbon dioxide in the decomposition of natural mannan (salep root) by certain bacteria was studied. It is certain that not all the carbon dioxide evolved came from the hemicellulose in the salep, but the mannan content was so large in proportion to the other constituents and the microörganisms have shown their abilities to decompose the mannan so readily, that one is safe in assuming that most of the carbon came from the decomposition of the mannan. The results of this experiment are given in table 15. The bacteria were active over an extended

period of time. This is shown by the fact that the rate of decomposition did not decrease very much toward the end of the incubation period.

The rate of decomposition of the xylan preparation obtained from corn cobs by various aerobic bacteria was also studied. The plan of the experiment was identical with that employed in the case of the mannan. Table 16 shows the accumulative evolution of carbon dioxide from xylan in the sand medium by pure cultures of the aerobic bacteria. A few organisms showed very rapid decomposition for a short period of time while the remainder of the bacteria showed a more uniform course of decomposition.

An attempt has been made to summarize the rate of decomposition of some of the hemicelluloses by the various aerobic bacteria. The average carbon dioxide evolution by these bacteria is shown in figure 1. The purified mannan was found to be decomposed more rapidly than the mannan in the untreated plant material, especially in the early stages of decomposition. The purified xylan was decomposed less rapidly and less extensively than the mannan.

A comparison of the extent of decomposition of the three hemicelluloses; namely, the mannan, xylan, and galactan, both in a purified form and in a natural state, in sand and in solution media is shown in figure 2. Since the bacteria tested were aerobic, greater decomposition was brought about in the sand than in the solution media. The galactan was found to be very resistant to the activities of these organisms. However, the mannans were attacked very readily, as shown by the fact that 85 per cent of this hemicellulose was decomposed in the sand medium. In the solution medium, the natural materials were decomposed to a greater extent than the purified preparations.

The soil was found to contain a large number of anaerobic bacteria capable of decomposing the various hemicelluloses, especially the unpurified materials, such as the mannan in the salep root. The galactan was found to be most resistant to the action of the anaerobic organisms. These bacteria were all spore-forming rods, producing abundantly various gases (hydrogen, methane, carbon dioxide, etc.) and organic acids (butyric, propionic, acetic, etc.). The fact that the composition of the gases and of the acids varied with the different organisms points to differences in the chemical processes involved in the decomposition of the hemicelluloses.

SUMMARY

Investigations dealing with the decomposition of various hemicelluloses by aerobic and anaerobic bacteria are reported.

Many aerobic bacteria were found to be able to decompose hemicelluloses, the differences observed being both qualitative and quantitative in nature.

Galactan was found to be more resistant to decomposition by aerobic bacteria than mannan and xylan.

In the case of aerobic bacteria, both the nature of the hemicellulose and the environmental conditions influenced markedly the quantities of hemicellulose decomposed.

Chemical purification appears to render hemicelluloses more resistant to decomposition. This is especially true of the anaerobic bacteria.

Organic acids were produced both by aerobic and anaerobic bacteria in the decomposition of hemicelluloses.

The anaerobic bacteria produced different gases in the decomposition of the hemicelluloses.

GENERAL SUMMARY

The results presented in these papers point definitely to the fact that the soil contains an abundant flora or fungi, actinomyces, aerobic and anaerobic bacteria, capable of decomposing various hemicelluloses.

The microörganisms decomposing the hemicelluloses are not so specific as those bacteria and fungi which are capable of decomposing cellulose. It is true, of course, that some organisms attack preferably one type of hemicellulose and some another; some of the hemicelluloses, like the galactans, are more resistant to decomposition than others, like the mannans, and probably also more than even the true cellulose. However, the decomposition of the most common hemicelluloses, such as the xylans and the other pentosans as well as the mannans, is not limited to any one special group or few specific types of bacteria and fungi, but can be brought about by a great variety of organisms found among a number of different types which are of common occurrence in nature.

As a result of this lack of specificity among the fungi and bacteria capable of attacking hemicelluloses, no attempt has been made to describe new types of organisms bringing about the decomposition of these carbohydrates, since hundreds of species could thus be readily isolated and described. The desirability of describing all of these species was questioned, especially because most of them did not represent any special physiological or morphological types. Of course, one cannot deny the existence of certain specific bacteria capable of attacking one type of hemicellulose in preference to others, as was found to be the case of certain pectin dissolving bacteria, agar-liquefying bacteria, bacteria decomposing capsular polysaccharides, etc. If such specific organisms are isolated, especially if they represent groups of possible economic importance, they should, of course, be studied and described in detail. This was found to be the case of a group of anaerobic bacteria which, as a result of the production of an abundance of organic acids and gases in the decomposition of hemicelluloses, may represent certain interesting phases of microbial physiology and possible economic utilization. These cultures have been reserved for a more extended study in the future.

The microörganisms bringing about the decomposition of the hemicelluloses require available sources of nitrogen for the synthesis of their microbial cell substance, just as cellulose-decomposing bacteria do. The ratio between the hemicellulose decomposed and the nitrogen consumed is about 30 to 1 with a variation of 20 to 40 to 1. In this respect, they resemble the fungi and repre-

sent an interesting phase of transformation of organic matter in soil, and formation of soil organic matter or soil humus.

The study of the transformation of uronic acid complexes in the decomposition of hemicelluloses by microörganisms is reserved for a future publication.

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STUDIES ON THE TRANSFORMATIONS OF IRON IN NATURE: III. THE EFFECT OF CO_2 ON THE EQUILIBRIUM IN IRON SOLUTIONS

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In a former publication in this series (11), conditions of equilibrium were considered in iron solutions upon which the following restrictions were placed:

First, that ferrous iron underwent reversible oxidation to ferric iron according to the equation:

$$4\ \overset{..}{Fe} + O_2 + 2\ \overset{.}{H} = 4\ \overset{...}{Fe} + 2\ \overline{OH}.$$
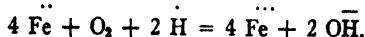
Second, that the solution was saturated with respect to the ferric iron in accordance with the equation:

$$[A_{Fe^{.}}]\ [A_{\overline{OH}}]^3 = K$$

With these restrictions and under atmospheric pressure of oxygen the following relationship was deduced:

$$\frac{[A_{\overset{.}{Fe}}]}{[A_{\overset{.}{H}}]^2} = C$$

Solutions of ferrous sulfate were allowed to remain in contact with air for 4 months. At the end of this time the concentration of Fe˙˙ was obtained from a determination of the total amount of iron present. The solutions were very dilute so that this was assumed to be equal to the activity of the ferrous ions. The activity of the hydrogen ion was determined from pH measurements. Under these conditions the average value of the foregoing ratio in a series of tests was found to be 4.8×10^3.

In the second article of the series (22) the rôle of microorganisms in the transformations of iron in nature was considered in the light of the equations developed. In attempting to evaluate the rôle of microorganisms it became apparent that CO_2 is of major importance.

In order that the influence of CO_2 may be better understood, it is necessary to see what effect this gas may have upon the aforementioned equilibrium. In the foregoing deductions, it was assumed that the solutions containing ions of iron would undergo a reversible oxidation when exposed to atmospheric oxy-

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gen. Because of the peculiarities of molecular oxygen when employed as an oxidizing agent, this assumption may not hold. To get a better picture of the true state of affairs it becomes necessary to avoid this assumption.

Equilibrium conditions can then be deduced by making use of the methods employed by Clark (4) and Kolthoff and Furman (13). They employed the fundamental equation of Nernst (1889),

$$E = -\frac{RT}{NF} \ln \frac{P'}{p},$$

where E is the potential difference between the metal and the solution, T the absolute temperature, N the valence of the ion, and F the Faraday, i.e., the quantity of electricity that is associated with one gram-equivalent, viz., 96,500 coulombs. R and F are constants, the values of which are known. P' is the solution pressure of the metal, and p is the osmotic pressure of the metallic ions. Transposing the natural logarithms to the Briggsian, the following is obtained:

$$E = -\frac{0\,0591}{N} \log \frac{P'}{p}$$

From this equation, they deduce the following:

$$E = +\,0.0591 \log \frac{A}{A_e}, \tag{A}$$

where A_e represents the electronic activity of the solution, a value which is a measure of the oxidation-reduction potential of the system. A is a constant so that equation (A) can be rewritten as follows:

$$E = A' - 0.0591 \log A_e \tag{B}$$

In a solution where ferrous iron is oxidized reversibly to ferric iron, the changes can be indicated by the following equation:

$$\overset{\cdots}{\text{Fe}} + e = \overset{\cdot\cdot}{\text{Fe}}.$$
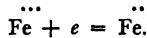
According to the mass law, this will give

$$\frac{[A_{\overset{\cdot\cdot}{\text{Fe}}}]\,[A_e]}{[A_{\overset{\cdots}{\text{Fe}}}]} = K$$

or

$$A_e = K\,\frac{[A_{\overset{\cdot\cdot}{\text{Fe}}}]}{[A_{\overset{\cdots}{\text{Fe}}}]}$$

Upon substituting this value of A_e in equation (B) there is obtained

$$E = A' - 0.0591 \log K\,\frac{[A_{\overset{\cdots}{\text{Fe}}}]}{[A_{\overset{\cdot\cdot}{\text{Fe}}}]}$$

or

$$E = A' - 0.0591 \log K + 0.0591 \log \frac{[A_{Fe^{\cdots}}]}{[A_{Fe^{\cdot}}]}.$$

The term $(A' - 0.0591 \log K)$ is constant for this system and can be called E_o. Then,

$$E_o = E + 0.0591 \log \frac{[A_{Fe^{\cdots}}]}{[A_{Fe^{\cdot}}]} \qquad \bullet \qquad (C)$$

Putting the E_o of the normal hydrogen electrode equal to O and using the sign convention adapted by the American Electrochemical Society, Peters (19) has determined the value of E_o (Fe$^{\cdots}$— Fe$^{\cdot\cdot}$) to be +0.743. Forrester (7) gives the value of +0.714. This value is accepted by Clark (4), and Kolthoff and Furman (13), and is probably the more accurate. Using this value we can then write.

$$E_h = + 0.714 + 0 0591 \log \frac{[A_{Fe^{\cdots}}]}{[A_{Fe^{\cdot}}]} \qquad (D)$$

By means of this equation it is possible to calculate the oxidation-reduction value of the solutions containing varying amounts of ferrous and ferric iron. It is also possible by means of this equation to determine the stable ratio of these two ions in solutions when the oxidation potential is fixed by some other system.

In a similar manner we can develop the equation of the oxidation-reduction potential of a system in which oxygen is in equilibrium with oxygen combined in the form of water, according to the equation:

$$O_2 + 4 \overset{\cdot}{H} + 4 e = 2 H_2O.$$
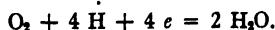
By applying the mass law, we get the following:

$$A_e = \frac{K}{[A_{O_2}]^{\frac{1}{4}} [A_H]}$$

Substituting this value of A_e in equation (B) we have

$$E = A' - 0.0591 \log K + 0.0591 \log [A_{O_2}]^{\frac{1}{4}} [A_{\overset{\cdot}{H}}]$$

or

$$E = E_o + 0.0591 \log [A_{O_2}]^{\frac{1}{4}} [A_H] \text{ at } 25°\overset{\cdot}{C}.$$

$E_{o(O_2 - H_2O)}$ has been calculated by G. N. Lewis (14) from the heats of formation of water from the elements. He obtained the value for E_o of 1.227 referred to the hydrogen scale. This value was obtained by using values for the heats of formation obtained from three independent sources. The value

should therefore be correct for systems in which there is a perfectly reversible exchange of electrons between the oxygen combined in water and molecular oxygen. We can then write:

$$E_h = + 1.227 + 0.0591 \log [A_{O_2}]^{\frac{1}{4}} [A_{\dot{H}}] \qquad (E)$$

If we assume that ferrous ions were oxidized reversibly by molecular oxygen, we can, by means of equations (D) and (E), calculate the equilibrium constant of this reaction.

$$4 \overset{..}{Fe} + O_2 + 4 \overset{.}{H} = 4 \overset{...}{Fe} + 2 H_2O.$$
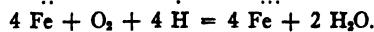
From this, the mass law will give

$$K = \frac{[A_{\overset{...}{Fe}}] [A_{O_2}]^{\frac{1}{4}} [A_{\dot{H}}]}{[A_{\overset{..}{Fe}}]}$$

At equilibrium the oxidation potentials of the systems indicated in equations (D) and (E) must be equal. Therefore,

$$E = + 0.714 + 0.0591 \log \frac{[A_{\overset{...}{Fe}}]}{[A_{\overset{..}{Fe}}]} = + 1.227 + 0.0591 \log [A_{O_2}]^{\frac{1}{4}} [A_{\dot{H}}].$$

From this,

$$- \log K = 8.64 = \log \frac{[A_{\overset{...}{Fe}}]}{[A_{\overset{..}{Fe}}] [A_{O_2}]^{\frac{1}{4}} [A_{\dot{H}}]}$$

and

$$K = 2.3 \times 10^{-9}.$$

If we further assume that the activity of the oxygen is directly proportional to its pressure, and put the activity equal to unity when the pressure is one atmosphere, then under constant pressure of oxygen the equilibrium conditions can be expressed as follows:

$$\frac{[A_{\overset{...}{Fe}}]}{[A_{\overset{..}{Fe}}]} [A_{\dot{H}}] = 2.3 \times 10^{-9} \qquad (F)$$

If, instead of assuming that the oxygen pressure is kept constant at the pressure of one atmosphere, we assume that it is kept constant at the pressure equivalent to its partial pressure in the atmosphere where there are 22 parts by volume of oxygen, we would then have,

$$[A_{O_2}]^{\frac{1}{4}} = (21)^{\frac{1}{4}} = .677.$$

Under these conditions,

$$\frac{[A_{\overset{...}{Fe}}]}{[A_{\overset{..}{Fe}}]} [A_{\dot{H}}] = 3.4 \times 10^{-9}. \qquad (G)$$

If then a solution containing ferrous ions were oxidized reversibly by the atmosphere, equation (G) would express the equilibrium conditions. As a result of placing this solution in contact with solid ferric hydroxide and employing the calculation used in the former paper (11), equation (G) reduces to

$$\frac{[A_{Fe}]}{[A_{\bar{H}}]^2} = 3.7 \times 10^{-3}. \qquad (H)$$

By multiplying both sides of this equation by the square of the dissociation constant of water one obtains the equation,

$$[A_{Fe}]\,[A_{O\bar{H}}]^2 = 3.7 \times 10^{-31}. \qquad (I)$$

We see then that this has led to the same general conclusion that was reached in the former paper; namely, that the oxidation has established the equivalent of a solubility product of ferrous hydroxide. The peculiarity of this solubility product is that if extra ferrous ions are introduced into this solution, they would not precipitate as ferrous hydroxide, but would be oxidized to the ferric state and be precipitated as the ferric hydroxide.

In a solution which was not undergoing such an oxidation Müller (18) determined the solubility product of ferrous hydroxide to be 1.64×10^{-14}, a value much larger than the one indicated by equation (I). The ultimate effect of such a reversible oxidation is then to reduce the solubility of the ferrous hydroxide by this enormous amount.

From equation (I) it is possible to see why a given amount of ferrous iron would be more completely oxidized under basic conditions than under acid conditions. Under acid conditions there could remain at equilibrium a relatively large amount of ferrous iron, whereas under basic conditions it would be almost completely removed from solution. In other words, under basic conditions ferrous iron is a much better reducing agent than under acid conditions. A mathematical demonstration of this fact has not been found in the literature, but in a qualitative way it has been observed that basic solutions of ferrous salts convert to the ferric form very rapidly, whereas acid solutions are oxidized by the oxygen of the air very incompletely and slowly (6, 9). Müller attempted to show this qualitatively by measuring the reducing power of ferrous solutions at various pH values by a direct measurement of the oxidation-reduction potential. In this study he showed that basic solutions of ferrous salts had a very high reducing value, whereas in acid solutions it was materially lower.

In the foregoing considerations it has been assumed that the oxidations by molecular oxygen were reversible. Under many conditions this may not be true. G. N. Lewis (14) found that he could not obtain the theoretical value of 1.227 in an oxygen-hydrogen cell, but by actual measurement found a lower value. Furman (8) by actual measurement found the e.m.f. of an oxygen-hydrogen cell to be 1.08 instead of the theoretical value of 1.227 as calculated

by Lewis. Montillon and Cassel (17) obtained the value of 0.99. These low values are undoubtedly due to the fact that in such a cell the oxidation is not entirely reversible. Under these conditions if we used the value $E_{o(O_2 - H_2O)}$ = 0.99 in the foregoing calculations, we would obtain the value of

$$[A_{Fe}] [A_{OH}]^2 = 2.8 \times 10^{-27}. \qquad (J)$$

Under such conditions then, the oxidation would not be as complete as that indicated above.

It is to be expected, therefore, that if ferrous solutions were left to undergo atmospheric oxidation, this oxidation might be even more incomplete. This has been shown to be the case in a previous publication (11). It was found that if inorganic solutions were allowed to come to an apparent equilibrium by means of atmospheric oxidation the following relations seemed to exist:

$$\frac{[A_{Fe}]}{[A_H]^2} = 4.77 \times 10^3 \qquad (K)$$

and

$$[A_{Fe}] [A_{OH}]^2 = 4.77 \times 10^{-25}. \qquad (L)$$

Under these conditions the oxidation is even less complete than that indicated under equation (J). We can also see from this that if a solution that had undergone this natural oxidation would be made to undergo a reversible oxidation, the solubility of the ferrous hydroxide would decrease from a value indicated by the product 4.77×10^{-25} to a value 3.5×10^{-31}. This fact may be of some importance in connection with the physiology of the iron bacteria. It will also help to explain the precipitation of iron by some of the heterotrophic bacteria.

All of the aforementioned considerations have been with inorganic iron solutions. In organic solutions other factors enter that may increase the amount of iron that can remain in solution. Complex ions of iron may be formed which may reduce the ionic concentrations of either the ferrous or the ferric iron to extremely low values so that in many cases no precipitation of the ferric hydroxide will occur. This may also change the oxidation-reduction potential.

Since iron in nature is so frequently transported in the form of the carbonate, it is also of importance to inquire into the chemistry of iron solutions where carbonate may play an important rôle. It will be of interest, therefore, to find the equilibrium conditions in a solution where the following conditions will be fulfilled:

The solution is saturated with respect to CO_2.
The ferrous iron is being oxidized by molecular oxygen reversibly and exists in equilibrium as indicated by equation (J).
The gaseous phase in equilibrium with the liquid contains the same partial pressure of oxygen as that found in air. Not much error will be introduced by assuming that

when a small amount of CO_2 is introduced to the gaseous phase, the partial pressure of the oxygen remains unchanged since its influence upon the equilibrium is the quarter power of its activity.

The temperature of the system is kept at 25°C.

The solution is saturated with respect to ferrous carbonate.

In this solution then, the amount of CO_2 dissolved is determined by the partial pressure of this gas. According to the data of Bohr (2), at 25° when the CO_2 is under a pressure of one atmosphere, pure water in contact with this gas will dissolve in it 0.759 cc. of the gas per cubic centimeter measured at 760 mm., pressure at 0°C. From this we can calculate the relationship between the partial pressure of the gas and its concentration in the solution, if we assume that Henry's law holds. Henry's law must be taken to mean that when the pressure of the gas is doubled, it doubles the concentration of the unionized portion of gas dissolved in the water.

If d = density of the gas at 0°C. and M = molecular weight of the gas, then $\dfrac{0.759 \times d \times 1{,}000}{M}$ must be equal to the sum of the concentrations of CO_2, H_2CO_3, HCO_3^-, and $\overline{CO_3}$. When the partial pressure of the CO_2 on the water is one atmosphere,

$$\frac{0.759 \cdot d \cdot 1{,}000}{M} - \text{moles of ionized portion} = \text{moles of unionized portion.}$$

Since in the determination of the dissociation constants of carbonic acid, the unionized portion is represented as H_2CO_3, the same condition is assumed in the following calculations. Then at P atmospheres of pressure of CO_2 we have

$$\frac{P \cdot 0.759 \cdot d \cdot 1{,}000}{M} - \text{moles of ionized portion} = [H_2CO_3]$$

or

$$0.0339\,P - \text{moles of ionized portion} = [H_2CO_3].$$

It will be necessary, then, to find out what portion of the 0.0339 moles of the CO_2 dissolved in free water at 25°C. are unionized. This can be calculated from the following equation:

$$H_2CO_3 \rightleftarrows \dot{H} + HCO_3^-$$
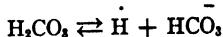
There will be $0.0339 - X$ moles of H_2CO_3, X moles of H, and X moles of HCO_3^-. From the mass law we can obtain an equation where X is the only unknown; namely,

$$\frac{X^2}{0.0339 - X} = K = 3.47 \times 10^{-7}. \quad (12)$$

From this equation X has the value of 0.00002. This is so small that it can be neglected. Therefore, we write the equation

$$0.0339\ P = [H_2CO_3]. \tag{M}$$

From this equation the relationship between the partial pressure of the CO_2 and the concentration of the hydrogen and $\overline{CO_3}$ ions can be found.

$$H_2CO_3 = 2\dot{H} + \overline{\overline{CO_3}}$$
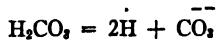
$$\frac{[\dot{H}]^2\ [\overline{\overline{CO_3}}]}{[H_2CO_3]} = K = 1.80 \times 10^{-17} \tag{12}$$

or,

$$[H_2CO_3] = \frac{[\dot{H}]^2 \cdot [\overline{\overline{CO_3}}]}{1.80 \times 10^{-17}}.$$

When this value of $[H_2CO_3]$ is put in equation (M) the following results:

$$P = [\dot{H}]^2 \cdot [\overline{\overline{CO_3}}] \cdot \frac{10^{17}}{1.80 \times .0339}$$

$$P = 1.64 \times 10^{18}\ [\dot{H}]^2\ [\overline{\overline{CO_3}}]. \tag{N}$$

In the solution described in the foregoing, one of the restrictions was that it should be saturated with ferrous carbonate. From the solubility product of this compound,·

$$[\ddot{Fe}]\ [\overline{\overline{CO_3}}] = K = 3.45 \times 10^{-11}.$$

Putting this value in equation (N), we obtain the following,

$$P = \frac{1 64 \times 10^{18}\ [\dot{H}]^2\ 3.45 \times 10^{-11}}{[\ddot{Fe}]} = 5.65 \times 10^7\ \frac{[\dot{H}]^2}{[\ddot{Fe}]}. \tag{O}$$

Another restriction on this solution was that it should be in a state of equilibrium as indicated by equation (I) viz.,

$$[\ddot{Fe}] \times [\overline{OH}]^2 = 3.7 \times 10^{-21}.$$

By dividing both sides of this equation by the square of the dissociation constant of water, we get

$$\frac{[\ddot{Fe}]}{[\dot{H}]^2} = 3.7 \times 10^{-2}.$$

When this is substituted in equation (O),

$$P = \frac{5.67 \times 10^7}{3.7 \times 10^3} = 1.54 \times 10^{10}.$$

In other words, such a solution is non-variant, as far as the pressure is concerned, at a constant temperature. It can exist only at a partial pressure of CO_2 of 1.54×10^{10} atmospheres, a value which is probably never attained.

If it is assumed that an oxidation-reduction potential exists, as was found in the solution of ferrous sulfate in which the ratio of the ferrous ions to the hydrogen ions squared was 4.77×10^3, the equilibrium pressure would be

$$P = \frac{5.67 \times 10^7}{4.77 \times 10^{-3}} = 1.17 \times 10^4$$

Even this value is very large.

Let us assume that the solution is maintained at a very low oxidation-reduction potential, but reversible however, so low in fact that the product $[Fe][OH]^2$ approaches the solubility product as a limit. In other respects the solution is not changed. In this case, in the limit where the solubility product is 1.64×10^{-14}, the ratio of the ferrous ions to the square of the hydrogen ions becomes 1.64×10^{14}, as found by Müller (18). In this case,

$$P = \frac{5.67 \times 10^7}{1.64 \times 10^{14}} = 3\,45 \times 10^{-7}$$

The partial pressure of the atmospheric CO_2 is 3.0×10^{-4}. In this last solution then we have one that may exist under certain conditions in nature. This will be discussed later.

From the foregoing equations, and equilibrium pressures of CO_2, predictions can be made as to what will happen if CO_2 is added or taken away from the system. If a small amount of CO_2 should be removed from the system in an attempt to lower its partial pressure, some of the solid ferrous carbonate in contact with the solution would dissolve in order to increase the concentration of the carbonate ion that had been lowered by the removal of the CO_2. This in turn would increase the concentration of the ferrous ion above a stable value. This would be compensated for by some of these being oxidized to the ferric state and being precipitated as ferric hydroxide. If CO_2 were continually removed from the system, the change would continue until all of the solid ferrous carbonate had dissolved. After that, further removal of the CO_2 according to the equation would result in a lowering of its partial pressure. The actual amount of ferrous iron in solution at the time when all solid ferrous carbonate is spent would depend on the acidity of the solution at that time. It can be readily seen from the above equations that the pressure of the CO_2 when the above solutions are in equilibrium is independent of the acidity. After all the solid ferrous carbonate is spent, however, a removal of the CO_2

would be accompanied by a decrease in the acidity in accordance with the fall of the partial pressure of the CO_2, according to the equation:

$$H_2O + CO_2 = H_2CO_3 = 2\overset{\cdot}{H} + \overset{--}{CO_3}.$$

As the acidity of the solution decreases, a precipitation of iron would occur as ferric hydroxide in accordance with the value of the ratio of ferrous ions to the square of the hydrogen ions. The value of this ratio depends, of course, upon the magnitude of the oxidation-reduction potential, as has been shown. If CO_2 should be added to a system that is in equilibrium with solid ferrous carbonate and ferric hydroxide and to one where there is a reversible oxidation-reduction by the O_2, this addition would bring about first a slight increase in the acidity, which would allow some of the ferric hydroxide to dissolve, and be reduced to the ferrous form. This would in turn supersaturate the solution with ferrous carbonate. A precipitation of this would then result. If CO_2 were continually added, this change would continue until all of the ferric hydroxide were dissolved. After this, further addition of CO_2 would increase the acidity and the concentration of the carbonate ion.

If CO_2 is added to a solution that contains no ferrous carbonate but is in equilibrium with solid ferric hydroxide, and a reversible oxidation-reduction is operative, such addition would cause an increase in the acidity, which in turn would dissolve some ferric hydroxide. Some of this would be reduced to the ferrous form. Further additions of CO_2 would continue this process until the solution became saturated with respect to ferrous carbonate.

By examining the equations from which was derived the equilibrium pressure of CO_2 at the various oxidation-reduction potentials, it can be readily seen that the pH of the various solutions cannot be determined from those equations. It would appear as though the pH might be changed without effect upon the system. This cannot be the case. It will be shown later that the pH can be changed within limits but not indefinitely. When solutions that contain no other ions but those furnished by the ferrous carbonate and hydroxide come to an equilibrium as has been indicated, the pH of the solution must be fixed. The pH, like the pressure of the CO_2, must depend upon the oxidation-reduction potential. From the following set of equations, its value can be determined. Since the solution must be electrically neutral, the sum of the charges must be equal to zero.

$$[\overset{\cdot}{H}] + 2\,[\overset{\cdot\cdot}{Fe}] - 2\,[\overset{--}{CO_3}] - [OH^-] = 0 \qquad (a)$$

$$[\overset{\cdot}{H}]\,[OH^-] = 10^{-14} \text{ (dissociation constant)} \qquad (b)$$

$$\frac{[\overset{\cdot}{Fe}]'}{[\overset{\cdot}{H}]^2} = K \text{ (from foregoing)} \qquad (c)$$

$$1.64 \times 10^{18} [\overset{.}{H}]^2 [\overline{CO_3}] = P_{CO_2} \text{ (from above)} \qquad (d)$$

When the oxidation potential is fixed and when a definite value is therefore placed upon the partial pressure of the CO_2, there remain then four unknowns, related by four independent equations from which all the variables may be determined. The most convenient way to solve this is to substitute in equation (a) for the ferrous, the carbonate, and the hydroxyl ions their equivalent in hydrogen ions as determined by equations (b), (c), and (d). There is obtained then a cubical equation that can be solved most readily by graphical means. Once having found the value of the hydrogen-ion concentration one can obtain the others readily from equations (b), (c), and (d). In table 1 are given the equilibrium values of the hydrogen-, the ferrous-, and the carbonate-ion concentrations calculated in this manner for the different states of oxidation discussed in the foregoing.

TABLE 1

Equilibrium values of the hydrogen-, Ferrous- and carbonate- ion concentration

NUMBER	$\dfrac{Fe}{H^2}$	P_{CO_2}	pH	H^+	Fe	$CO_3{}^{--}$	$Fe(HCO_3)_2$	TOTAL Fe
1	$3\ 5\ \times 10^{-2}$	$1\ 54 \times 10^{10}$	2 57	$2\ 71 \times 10^{-3}$	$2\ 56 \times 10^{-8}$	$1\ 28 \times 10^{-3}$	$4\ 9 \times 10^2$	490
2	$4\ 77 \times 10^3$	$1\ 17 \times 10^4$	4 61	$2\ 45 \times 10^{-5}$	$2\ 86 \times 10^{-6}$	$1\ 19 \times 10^{-5}$	$3\ 4 \times 10^{-3}$	$3\ 4 \times 10^{-3}$
3	$1\ 64 \times 10^{14}$	$3\ 45 \times 10^{-7}$	9 50	$3\ 16 \times 10^{-10}$	$1\ 63 \times 10^{-5}$	$2\ 11 \times 10^{-6}$	$1\ 0 \times 10^{-14}$	$1\ 63 \times 10^{-5}$
4	$4\ 77 \times 10^3$	$3\ 00 \times 10^{-4}$	6 15	$6\ 89 \times 10^{-7}$	$2\ 26 \times 10^{-9}$	$3\ 36 \times 10^{-7}$	$1\ 6 \times 10^{-13}$	$2\ 26 \times 10^{-9}$
5	$3\ 5\ \times 10^{-2}$	$3\ 00 \times 10^{-4}$	6 15	$6\ 89 \times 10^{-7}$	$1\ 66 \times 10^{-14}$	$3\ 36 \times 10^{-7}$	$1\ 2 \times 10^{-18}$	$1\ 67 \times 10^{-14}$

In table 1 the amount of the unionized ferrous bicarbonate was determined as follows: From equation (M) the unionized carbonic acid is related to the pressure of the carbon dioxide as follows:

$$P \cdot .0339 = [H_2CO_3]$$

At a definite pressure then, from this equation, it is possible to calculate the CO_2 in the solution. From the table given by Smith (21) it is possible to find the amount of ferrous bicarbonate in solution. In order to get values for the pressure above and below those given in Smith's data, a straight line relationship was assumed to exist between the dissolved CO_2 and the concentration of the ferrous bicarbonate. The amount of ferrous bicarbonate was then obtained from this relationship.

The values in this table are not to be looked upon as accurate values from which quantitative deductions can be drawn. The various assumptions that have to be made in deducing these values introduce errors in the more concentrated solutions, that is, solutions which are under one or more atmospheres of CO_2. In the dilute solutions, they will be more accurate. The values given are to serve rather as indicators, in a qualitative way, of the types of changes that may take place under various conditions.

By making use of the equations and calculated values expressing the various equilibrium conditions, developed in the foregoing, it will be possible to draw conclusions regarding the following points:

Conditions which will favor the iron carrying capacity of water.
Conditions under which iron will precipitate in nature due to purely chemical reactions, in the absence of microbial activity.
Conditions under which microbial activity may be responsible directly or indirectly for the solution of iron and its reduction from the ferric to the ferrous form.
Conditions under which iron may be precipitated in nature by microörganisms.
Conditions under which microbial activity may bring about the precipitation of ferrous carbonate.

CONDITIONS WHICH WILL FAVOR THE IRON CARRYING CAPACITY OF WATER

It can readily be seen from table 1 on the equilibrium states in ferrous carbonate solutions that as the acidity increases, the iron content also increases. In nature about the only way in which the acidity can be increased to any great extent in natural waters is by the addition of CO_2. This naturally makes the acidity of the water dependent upon the properties of carbonic acid. By examining the equation,

$$\frac{[Fe]}{[H]^2} = K,$$

it would appear that the larger this ratio the more iron will be held in solution. This would be strictly true if the acid that furnished the hydrogen ions were a strong acid and if the salt of iron formed by this acid be very soluble. This is not true with carbonic acid, however. Because ferrous carbonate has a rather low solubility product, because the carbonic acid is a weak acid, and because the bicarbonate of iron is readily soluble, it happens that the most favorable conditions for maintaining a high iron content in the water is to have a comparatively low value for the ratio of ferrous ions to the square of the hydrogen ions. Under these conditions only will the concentration of the ferrous ions in the solution be suppressed low enough to allow a sufficiently high carbonate-ion concentration to maintain an acid solution. When the aforementioned ratio is high it permits such a high ferrous-ion content in the solution that any attempt to increase the carbonate-ion content of the solution by increasing the pressure of CO_2 will result only in a precipitation of ferrous carbonate. Thus we see from table 1 that when the oxidation-reduction potential is high as indicated by an equilibrium ratio of

$$\frac{[Fe]}{[H]^2} = 3.5 \times 10^{-3},$$

it is possible to increase the CO_2 pressure on the system to the very high pressure of 1.54×10^{10} atmospheres before any precipitation of ferrous carbonate

would take place, thus making it possible to increase the acidity to a point where a large amount of iron can be held in the solution. It is further seen that when the oxidation-reduction potential reaches the very low value where it permits the high stable ratio of

$$\frac{[Fe]}{[H]^2} = 1\ 64 \times 10^{14},$$

the pressure of CO_2 cannot be increased above the low value of 3.45×10^{-7} atmospheres before a precipitation of ferrous carbonate would take place. This would make it impossible under these conditions to increase the acidity high enough with carbonic acid to allow the solution to hold more than a mere trace of iron. It is to be noted, however, that at a definite pressure of CO_2 it is possible to increase the iron content of the solution by decreasing the oxidation-reduction potential to an optimum value. Thus we see that when the CO_2 pressure is 3.00×10^{-4} atmospheres, more iron is held in the solution when the stable ratio of

$$\frac{[Fe]}{[H]^2} = 4.77 \times 10^3,$$

that when this ratio is forced to the lower value of 3.5×10^{-3}. If this ratio could be increased above the former value, which would be equivalent to decreasing the oxidation-reduction potential, it would soon increase the ferrous-ion concentration to a point where the ferrous carbonate would precipitate. Decreasing the oxidation potential still more would not further increase the concentration of the ferrous ion. This permits the rather definite conclusion that in systems where there exists a reversible oxidation-reduction for a given pressure of CO_2, there is a definite oxidation-reduction potential which will allow the maximum amount of iron to remain in solution. If the oxidation-reduction potential is changed in either direction from this value the iron content will be decreased. If the oxidation-reduction potential is increased, ferric hydrate will precipitate, and if it is decreased, ferrous carbonate will precipitate.

CONDITIONS FAVORING THE PRECIPITATION OF FERRIC HYDRATE FROM CARBONATED WATER IN THE ABSENCE OF MICROBIAL ACTIVITY

From the equations developed it can be readily seen that ferric hydrate in natural waters can be precipitated either by coming in contact with an atmosphere with a lower pressure of CO_2 than the CO_2 pressure of the solution; or by coming in contact with an environment of higher O_2 pressure, which in turn would increase the oxidation-reduction potential of the solution; or by undergoing both of these changes. Under natural conditions where iron is transported as the bicarbonate in underground waters it is difficult to estimate what

the actual oxidation-reduction potential might be. By examining the table 1 representing the equilibrium values, it can be seen that when any solution that is in equilibrium with ferrous carbonate and ferric hydroxide comes to the surface where it will be subjected to the atmospheric pressure of CO_2 and O_2, a large percentage of the iron carried by the water will be precipitated as ferric hydrate. Thus we see that solutions 1 and 2, which have an equilibrium pressure of CO_2 greater than the CO_2 pressure of the air, lose most of their iron when they finally reach the state indicated by solution 4. The oxidation-reduction potential of solution 1 is greater than that produced by the oxygen of the air, while the corresponding potential of solution 2 is the same as that produced by the air. Since in both of these solutions there is no increase in the oxidation-reduction potential as they pass from state 1 or 2 to 4, it must be concluded that the only cause for the precipitation here is the loss of CO_2 from the solutions as they pass from state 1 or 2 to 4. It is easy to conceive how this change would take place. As the CO_2 escapes from these solutions the acidity naturally decreases. This decrease in the H makes it necessary for the $Fe^{..}$ iron to be oxidized to the ferric state and to be precipitated as the ferric hydroxide in order to maintain the stable ratio of $\dfrac{[Fe^{..}]}{[H]^2}$ allowed by the oxidation-reduction potential of the solution. We see that in both of these solutions practically 100 per cent of the iron can be precipitated without the aid of any microorganism. It is not likely that the precipitation would entirely cease when it reached the state indicated by solution 4, when only 2.26×10^{-9} moles per liter of iron remain per liter, but any oxidation and precipitation taking place beyond this point would be so slow as to be of no importance in natural transformations. It is likely that the precipitation would be very slow before the concentration of the ferrous iron reached the value of 2.26×10^{-9} moles per liter. Theoretically the change should continue to state 5 with the very low value of iron concentration of 1.67×10^{-14} moles per liter. The fact that the oxidation of this type by molecular oxygen is not entirely reversible will prevent natural waters from reaching this very low value of iron content by pure chemical reactions in the absence of microbial activity.

In case of solution 3, when it changes to state 4 and decreases its iron content from 1.63×10^{-5} to 2.26×10^{-9} moles per liter, the cause of the precipitation is not the loss of CO_2. In this case the CO_2 tension of the liquid is less than that of the air. There is a marked increase in the oxidation-reduction potential that takes place as the conditions are changed from state 3 to 4. In this case, then, the increased oxidation-reduction potential must be considered as the cause of the precipitation of the iron.

In many cases there may be underground waters existing that are in a state intermediate between those found in solutions 2 and 4. Under those conditions, when these waters come to the surface, there will not only be a decrease in the CO_2 pressure, but there will also be an increase in the oxidation-reduction potential. In such cases the precipitation would be caused by both the loss of CO_2 from the water and by the increased oxidation-reduction potential.

In all of these cases then, it can be seen that nearly 100 per cent of the iron can be precipitated as the ferric hydrate by pure chemical reaction without the aid of microorganisms.

It is also theoretically possible to effect the precipitation of the iron by an increase in the alkalinity of the water other than that caused by the loss of CO_2. This can be done by having the water come in contact with the alkaline material or be diluted by alkaline solutions. Under natural conditions one way in which this is likely to happen is when underground water containing iron comes into contact with soil or water containing bicarbonates of strong alkalies, such as sodium or calcium. When the acidity is changed in this way without changing the CO_2 tension of the solution or the oxidation-reduction potential, the precipitation is caused exclusively by the change of reaction. The new lower value of H will naturally bring about a decrease in the Fe content in order to maintain the stable ratio of $\dfrac{[\text{Fe}^{\cdot\cdot}]}{[\text{H}]^2}$ under those conditions. The ferrous iron content would be decreased by being oxidized to the ferric form and precipitated as the ferric hydroxide. Such precipitation may be responsible for certain of the "hard-pan" formations occurring in certain soils. These formations, in the light of the foregoing deductions, can easily be explained by the last mentioned method of iron precipitation resulting from underground iron-bearing water coming into contact with lime-bearing water or calcareous soil near the surface. Such precipitation may also follow microbial activities. These have been considered in a former paper on the effect of heterotrophic bacteria on iron precipitation (22).

CONDITIONS UNDER WHICH MICROBIAL ACTIVITY MAY BE RESPONSIBLE FOR THE
SOLUTION OF IRON AND ITS REDUCTION FROM THE FERRIC TO THE
FERROUS FORM

From the foregoing theoretical considerations it has been pointed out that the ratio of the ferrous-ion concentration to the square of the hydrogen-ion concentration is dependent upon the oxidation-reduction potential, provided that the oxidation is effected by molecular oxygen. The ratio increases with a decrease in the oxidation-reduction potential. An increase in acidity of the solution and a lowering of the oxidation-reduction potential should then favor the solution and reduction of the iron. In many cases the acidity may be due to carbonic acid. Too high concentrations of this acid may limit the amount of iron that can be held in solution, as has been pointed out. As long as the amount of carbonic acid is relatively small only the oxidation-reduction potential and the acidity need be considered.

Solutions in which bacteria are growing, even though they be under aerobic conditions, should, in most cases, favor the solution of ferric iron and its reduction to the ferrous form. This should be particularly true if the solution becomes more acid as a result of the bacterial growth. This phase of the problem was considered in detail in a former publication in which experiments

and data are given showing how microbial activity brings about the reduction of ferric iron to the ferrous form.   The condition that favors the solution of iron from its salt and oxides should also favor the solution of free iron from the metallic state.   Grant (10) found this to be the case in some instances in the corrosion of iron in the soil.   He found that bacterial activity greatly increased the rate at which the iron disappeared.

TABLE 2

Effect of bacterial activity upon the dissolving action of liquids upon metallic iron

SOLUTION	pH	REDUCED IRON	TOTAL IRON
		mgm per 100 cc	*mgm per 100 cc*
Part A. Iron filings added before sterilization			
Dextrose uninoculated	3 46	Trace	Trace
Dextrose uninoculated	3 54	Trace	Trace
Dextrose inoculated	6 40	190	180
Dextrose inoculated	6 13	224	225
Peptone uninoculated	6 70	0	0
Peptone uninoculated	5 60	0	0
Peptone inoculated .	5 40	16	18
Peptone inoculated	5 50	14	15
Part B. Iron filings after sterilization			
Dextrose uninoculated	5 66	Trace	Trace
Dextrose uninoculated	5 86	Trace	Trace
Dextrose inoculated	6 20	130	130
Dextrose inoculated	6 23	121	120
Peptone uninoculated	3 70	None	None
Peptone uninoculated	3 72	None	None
Peptone inoculated	5 50	10	10
Peptone inoculated	5 52	10	10

SOLUTION	pH	IRON
Part C No iron filings added		
Dextrose uninoculated	6 00	None
Dextrose inoculated	2 40	None
Peptone uninoculated	6 70	None
Peptone inoculated	5 60	None

For this experiment media were prepared having the same composition as those used in the experiment on ferric hydroxide.   To three flasks containing 1 per cent dextrose, iron filings were added, as was also done to three flasks containing 1 per cent peptone.   To four flasks, one containing dextrose and one peptone, no iron was added.   All the flasks were sterilized in the autoclave at 15 pounds steam pressure for 30 minutes.   Two of the dextrose flasks containing iron filings and one containing no iron were inoculated with soil and incu-

bated under anaerobic conditions, as used in the previous experiments, for 3 weeks. A corresponding set of the peptone flasks were treated in the same way. All the others remained uninoculated as controls.

Since it was conceived possible that the high temperature used in sterilization might in itself dissolve a considerable portion of the iron, a similar set of flasks was prepared that was sterilized first and the iron filings then added prior to incubation. These flasks were inoculated in the same order as the foregoing. Good growth resulted in all the inoculated flasks. Subsequent to incubation, the solutions were analyzed for soluble iron and acidity by pH measurements. The results of this experiment are given in table 2.

Here also it can be seen that under conditions where bacteria are growing and forming anaerobic conditions, a considerable amount of iron is dissolved and held in solution in the reduced form. This was particularly striking in the dextrose cultures. Peptone cultures do not show true results; because in the breakdown of the peptone rather large amounts of H_2S are formed which precipitate the iron as the sulfide. The inoculated peptone cultures showed a large amount of black precipitate. Not a very striking difference occurs between the cultures where the iron was added before and after sterilization. In the sterile cultures a considerable amount of iron oxide had formed, but very minute quantities remained in solution. In the dextrose cultures that contained the large amounts of iron, a very rapid oxidation and precipitation occurred after the solutions were exposed to the atmosphere. This precipitation was so rapid that it was necessary to analyze the solutions as soon as the stoppers were removed. In two instances, because of an unavoidable delay, some precipitation had occurred before the sample for the total iron had been taken. This accounts for the low results in these two cases. After the solutions had remained open to the atmosphere not more than 5 minutes, an extremely heavy precipitate of ferric hydroxide had formed.

From the results of this experiment it is easy to see that bacterial activity may become a very important factor in soil corrosion. By the action of bacteria a large amount of iron can remain in the solution and thus favor the solution of the metallic iron (22). Furthermore, bacterial activity will prevent the formation on the surface of the iron of an oxide film which is regarded as a protective film that retards corrosion.

CONDITIONS PREDISPOSING THE PRECIPITATION OF IRON

The effects of the heterotrophic bacteria on the precipitation of iron by changing the environmental conditions have already been considered, in a former publication.

Bacteria may be concerned in the precipitation of that iron in nature which is carried in solution in underground waters as ferrous bicarbonate, when this water comes to the surface. According to equations that have been developed previously, it was shown that rather large amounts of iron could be held in solution in underground water when the oxidation-reduction potential was

low. It was also shown that a very small amount of this iron could remain in solution under aerobic conditions. This would tend to suggest that it is unnecessary to assume any microbial activity to account for the precipitation of iron from such waters when they come to the surface. Undoubtedly, practically all the iron would precipitate without microbial activity. The fact remains, however, that in all the so-called iron springs, the ferric hydrate is found in the sheaths or bands of certain bacteria. This has led some bacteriologists to the assumption (3) that specific iron bacteria must be able to carry out precipitation of iron that would not occur in their absence. The equilibrium conditions that have been developed show conclusively that where large amounts of iron are in solution as the ferrous bicarbonate, this assumption is erroneous.

In table 1 it was shown that ferrous bicarbonate solutions, upon coming to the surface to atmospheric environment, could undergo a more or less rapid transformation until the iron content was reduced to about 2.3×10^{-9} moles per liter. It was further shown that this could be further reduced to 1.67×10^{-14} moles per liter if the oxidation were made reversible at the atmospheric pressure of oxygen. If there are bacteria, then, that can, either by enzymes or by their vital activity, bring about reversible oxidations, such bacteria could reduce the iron content of water from the value of 2.3×10^{-9} moles per liter to the value of 1.67×10^{-14} moles per liter. It is not to be assumed that this is a transformation that could not occur spontaneously. It must be pointed out that the first value was obtained by actual experiment in determining the extent of oxidation by the oxygen of the air in a solution of ferrous sulfate. In these experiments, the solutions of ferrous sulfate were allowed to stand for 4 months. It cannot be assumed that the reactions had gone to completion at that time. The ultimate end point after an infinite time should be the value of 1.67×10^{-14}. Reduction of the iron content from the value of 2.3×10^{-9} to 1.67×10^{-14} would be extremely slow. Bacteria that can increase the oxidation-reduction potential may speed up the reaction and in this way be of considerable importance in iron precipitation in low iron-bearing waters. It is only reasonable to assume that bacteria that can increase the oxidation-reduction potential of a solution will be concerned with iron precipitations in solutions where oxidation and precipitation by the atmospheric oxygen may be fairly rapid. If they create an oxidation-reduction potential that is much greater in the immediate neighborhood of the bacterial cells than in the portions of the solution away from the cells, the chances of iron being oxidized in or near the cells are much greater than away from the cells. The result of this will be that practically all of this iron will be oxidized in or near these organisms even in solutions where the iron content is considerably above 2.3×10^{-9} moles per liter. Such bacteria, then, can be regarded as taking advantage of a chemical reaction that is occurring spontaneously. It would not be necessary to assume that such organisms make use of the energy liberated by this reaction. It is possible, however, that in some cases, this energy is utilized by the organ-

isms in their growth.   In the case of the autotrophic iron bacteria, there must be actual dependence upon this reaction (3).   These autotrophs can be regarded as heterotrophs that have been living in the environment of this reaction until they have finally degenerated to a point where they are actually dependent upon the oxidation of ferrous to ferric iron for their energy.

In this connection it will be of interest to inquire into the usual iron content of water in places where iron bacteria are found in nature.   Analyses were made of waters from four springs in which an abundant development of iron bacteria was found.   Samples were collected in 300-cc. Erlenmeyer flasks.   These were completely filled and tightly stoppered so as not to allow any great change to occur before they reached the laboratory.   Samples were taken as close as possible to the point where the water first became exposed to the air and also just below the point where any development of iron precipitating bacteria was visible.   In one case, samples were collected intermediate between these two points and also at a point some distance below the last visible sign of iron precipitate.   The acidity was determined by pH measurements made by means of a Leeds & Northrup type "K" potentiometer with a calomel half cell and Hildebrandt hydrogen electrodes.

The iron content was determined [see San Yin Wong (20)] by a colorimetric method by evaporating 25-cc. samples to which had been added 1 cc. of concentrated H_2SO_4 until the solution fumed with SO_3 vapors.   A 1-cc. portion of a 10 per cent solution of potassium chlorate was then added and the resulting solution again heated until SO_3 fumes again appeared.   A second portion of 0.1 cc. of potassium chlorate was then added to assure the complete breakdown of any organic matter which might be present.   After again being heated until SO_3 fumes appeared, the samples were made up to a volume of 15 cc. and to this was added 10 cc. of a 10 per cent solution of potassium sulfocyanate. A 1-cc. sample of a standard iron solution containing .0001 gm. of iron per cubic centimeter was treated in the same way.   By comparing the color of the standard with the unknowns in a Dubosk colorimeter the iron content of the various samples was determined.   In some of the samples that had very small amounts of iron it was found necessary to use 50-cc. samples of the water for the determination of the iron.   In all the determinations it was found that the red color remained apparently unchanged for at least 10 minutes.   The results of these analyses are shown in table 3.

Spring "a" was a very large spring found on the shores of the St. Croix river.   In this spring, sample 1 was obtained just as the water emerged to the surface.   It is not likely that any of the iron had precipitated from the water before this sample had been taken.   Sample 3 was taken at the point where the iron precipitating bacteria seemed to cease developing.   Sample 2 was taken at a point intermediate between 1 and 3.   Sample 4 was taken at a point where the water from the spring had traveled about one quarter mile.   The point where sample 3 was taken was about midway between the points where samples 1 and 4 were taken.   A considerable amount of organic matter, par-

ticularly dead leaves, had been deposited in the water between the points where samples 3 and 4 were taken. This may have interfered with further precipitation of iron.

Spring "b" was a somewhat smaller spring also found near the St. Croix River. In this case a sample could not be obtained before any iron had precipitated, as the water passed through horizontal crevices in the rocks in which some of the iron was precipitating. Sample 1 was taken just where the water emerged from the crevice. Sample 2 was taken at the point where further precipitation was not visible. Sample 3 was taken after the water had moved about 100 feet beyond where sample 2 was taken.

Spring "c," also emptying into the St. Croix River, and spring "d," emptying into the Mississippi River, were the same type of springs as "b," and the same difficulty in getting the first sample was experienced. In both these

TABLE 3

The iron content and pH of natural iron springs

SAMPLE	pH	Fe	LOG Fe	$LOG\frac{[Fe\cdot]}{[H\cdot]^2}$	$\frac{[Fe\cdot]}{[H\cdot]^2}$
		moles per liter			
1 a	6 8	17 4 $\times 10^{-5}$	6 24–10	9 8	7 $\times 10^9$
2 a	5 0	2 28 $\times 10^{-5}$	5 36–10	5 4	2 $\times 10^5$
3 a	4 8	1 73 $\times 10^{-5}$	5 24–10	4 8	7 $\times 10^4$
4 a	5 5	1 00 $\times 10^{-5}$	5 00–10	6.0	1 $\times 10^6$
1 b	6 0	10 16 $\times 10^{-5}$	6 00–10	8 0	1 $\times 10^8$
2 b	4 7	1 18 $\times 10^{-5}$	3 07–10	4 5	3 $\times 10^4$
3 b	4 8	0.49 $\times 10^{-5}$	4 69–10	4 3	2 $\times 10^4$
1 c	5 5	9 88 $\times 10^{-5}$	5 99–10	7 0	1 $\times 10^7$
2 c	4 6	1 16 $\times 10^{-5}$	5 06–10	4 0	1 $\times 10^5$
1 d	5 2	4 10 $\times 10^{-5}$	5 61–10	6 0	1 $\times 10^6$
2 d	4 6	1 82 $\times 10^{-5}$	5 25–10	4 5	3 $\times 10^4$

springs the second sample was taken below the point where no further visible precipitation of iron occurred.

In table 3 the ratio $\frac{[Fe\cdot]}{[H\cdot]^2}$ was calculated from the value of the iron content and pH value found. It has been shown that this ratio can be regarded as a measure of the oxidation-reduction potential. In the table these values indicate what the oxidation-reduction potential would have to be in order that the solutions with the corresponding amounts of iron might be stable. Under atmospheric conditions the apparent stable value of $\frac{[Fe\cdot]}{[H\cdot]^2}$ was found to be 7.3 $\times 10^8$. It is apparent that in the analysis in all the springs tested, the iron precipitated rather rapidly until the ratio $\frac{[Fe\cdot]}{[H\cdot]^2}$ approached a value near 10^4. There is a little discrepancy in the case of sample 4 a. This can be explained

on the basis of the organic matter present in the water and which would tend to prevent precipitation of iron, either because of the formation of complex ions or the development of heterotrophic bacteria that would tend to prevent precipitation of the iron (22).

It is apparent from the analyses that the iron precipitation was brought about by the increased oxidation-reduction potential effected by the atmospheric oxygen. The ferrous iron was oxidized to the ferric and this was precipitated as the hydroxide, leaving the carbonic acid, which increased the acidity. The escape of the CO_2 from the solution was apparently slower than the oxidation and precipitation of the iron. There is an indication from the table that after the rapid precipitation had ceased there is a gradual decrease in the acidity as the CO_2 escapes. This is accompanied by a slow oxidation of iron. Since the iron contents of these solutions are not reduced below a point that would be reached by pure chemical oxidation in the absence of microbial activity, no great importance can be attached to the bacteria that are associated with the precipitation of iron in iron springs. It may be that the iron is oxidized faster in or near these bacteria but the fact that they do not decrease the iron content below a point where it would go in their absence speaks against the formation of any oxidizing enzymes that have been attributed to these organisms. These organisms can be regarded as profiting from a chemical reaction that would reach the same end point in their absence.

CONDITIONS WHICH WILL FAVOR THE PRECIPITATION OF FERROUS CARBONATE

It has been shown, in considering the system (CO_2, H_2O, ferrous and ferric iron), that, under certain conditions, a precipitation of ferrous carbonate may occur. Conditions which would favor such precipitations were found to be a low oxidation-reduction potential with a CO_2 pressure above the corresponding equilibrium pressure. It has been shown that when the oxidation-reduction potential is forced to a very low value, the corresponding equilibrium pressure of CO_2 is rather low.

It is not unreasonable to expect, therefore, that microorganisms may effect such precipitation. Heterotrophic bacteria creating anaerobic or semianaerobic conditions in environments where they produce rather large quantities of CO_2 should favor the precipitation of ferrous carbonate in media in contact with iron or its oxides or salts. Tache (24) found that, in bogs where a high bacterial development was taking place, ferrous carbonate accumulated in the anaerobic portions. Van Hise (25) shows that large deposits of ferrous carbonate, which he assumes are formed under conditions of low oxygen tension, are found in nature. In a series of flasks filled with a medium containing dextrose, 1 per cent; NH_4NO_3, 0.05 per cent; $MgSO_4 \cdot 7H_2O$, 0.01 per cent; and iron filings, and another series containing the same ingredients with the exception that 1 per cent peptone was substituted for the dextrose, it was found that a white precipitate formed in the bottom of those flasks which were inoculated

with soil and incubated under anaerobic conditions. This material effervesced when treated with HCl. Since the calcium and magnesium content of this medium was very low there remained only the iron to account for this carbonate. The precipitate was therefore considered to be ferrous carbonate. Because of the rapid decomposition and oxidation of this material when the flasks were opened to the atmosphere, no attempt was made to analyze the precipitate quantitatively.

CONDITIONS THAT MUST BE FULFILLED FOR THE CULTIVATION OF THE IRON BACTERIA

A few bacteriologists have reported that they have cultivated under laboratory conditions bacteria that depend for their energy upon the oxidation of ferrous iron to ferric iron. Notable among these are Winogradsky (26, 27) and Lieske (15). Others have reported that they could not get a development of these organisms. The work of Suessenguth (23) is of interest in this connection. He reports that he could not confirm the results of Lieske. Since these organisms must be regarded as being dependent upon a chemical reaction that is occurring spontaneously, it is possible to point out the conditions that must be fulfilled in order to develop these organisms under laboratory conditions.

In any medium where these bacteria are to be grown, the ferrous iron content must be maintained at a concentration where it is undergoing atmospheric oxidation. This means that in order to maintain growth, a natural iron spring has to be imitated or fresh amounts of ferrous carbonate have to be added to the solution continuously. These requirements are not easily carried out under conditions where pure cultures have to be maintained. I have tried both of these methods of cultivating iron bacteria, but in no case could an active culture be maintained. In every case the solutions became noticeably acid, so that the lack of bacterial development could be explained on the basis of high acidity. An abundant precipitate was obtained in every case with or without the inoculation of the media with material from natural iron springs.

Lieske (15) and Bass-Becking (1) have reported that they could cultivate these bacteria by suspending iron filings on the surface of synthetic media containing no organic matter. It is of course possible that metallic iron can supply enough dissolved iron to make conditions favorable for their growth. If this is to be done, ideal conditions for the slow oxidation of iron by free atmospheric oxidation must be maintained. The iron used will have to be free from all impurities that tend to catalyze the oxidation of ferrous iron to the ferric state. Otherwise there will result only an accumulation of rust on or near the filings and no or too few ferrous ions will be furnished to the medium to support growth. In this connection it is of interest to note that Bass-Becking (1) reports that small amounts of copper as an impurity in the iron filings interfered with the development of the bacteria. Maquenne (16) found

that copper catalyzes the oxidation of ferrous iron to ferric iron. This may account for the poisonous effect of the copper. Many attempts were made by the author to cultivate the so-called iron bacteria, employing the medium advocated by Lieske (15) and Bass-Becking (1), but in no case were successful cultures obtained. In these attempts both impure iron filings and filings prepared from the purest iron obtainable were used. In spite of all precautions, there must have been chemical catalyzers present that brought about an oxidation that was too rapid. Lieske states that the iron bacteria appear in about 4 days. In all of the author's attempts a heavy precipitate of ferric hydroxide appeared in 1 and 2 days. In 4 days the process seemed to be practically complete. No indication of bands of iron could be detected microscopically in the accumulated precipitate. In stained preparations many structures appeared that looked like bacteria and that retained the violet dye of the gram stain. Since these same structures were found by preparing a slide of iron filings it was concluded that these were not bacteria but only iron filings that stuck to the slide. It does not seem possible to maintain the autotrophic iron bacteria in pure culture with sufficient degree of certainty to allow extensive studies of their physiology.

It appears that the definite conclusion can be drawn that, in order to cultivate autotrophic iron bacteria, it is necessary to create such conditions in a medium where iron is continuously undergoing spontaneous oxidation from the ferrous to the ferric state that the bacteria in question can live by chance upon this reaction as a source of their energy.

SUMMARY

Equilibrium conditions have been considered in iron solutions under the influence of atmospheric oxygen and carbon dioxide, and equations have been developed expressing the equilibrium conditions.

Some of the activities of microorganisms associated with solution and precipitation as well as oxidation and reduction of iron are considered in the light of equations developed. The following observations were noted.

Under anaerobic conditions in dextrose or peptone media, heterotrophic microörganisms may dissolve metallic iron. They will also dissolve and reduce iron present as ferric hydroxide. These changes result from a decrease in the oxygen pressure and the formation of acid. These transformations may occur even at reactions close to neutrality.

Precipitation of ferrous carbonate may result under anaerobic conditions when CO_2 is formed by the breakdown of organic compounds.

Activity of iron bacteria appears to occur only under environmental conditions favorable to spontaneous oxidation by chemical agencies.

The iron content of natural water in iron springs is not reduced by the iron bacteria to a concentration below which it will go by a pure chemical reaction, under identical conditions.

The solution and precipitation of iron in nature are seen to be associated with equilibrium conditions which depend upon the oxygen tension, carbon dioxide tension and acidity, and the presence of organic compounds. These conditions may be modified very extensively by bacterial activity. Careful analysis of the activities of the so-called iron bacteria indicates

that their contributions to transformations of iron are confined solely to its precipitation. It is of great importance to note that their activity appears to occur only under those conditions in which the iron precipitates spontaneously, that they do not carry on the reaction beyond the degree arrived at by spontaneous precipitation. On the other hand it has been shown that bacteria not recognized as iron bacteria greatly modify their environmental conditions so as to bring about either the solution or precipitation of iron. Their effect upon the solution of iron can hardly be overemphasized in that they can effect changes favoring solution which do not occur spontaneously. Although the deposition of the oxide is seen to occur readily as a spontaneous precipitation, the deposition of the carbonate in nature indicates that the heterotrophic bacteria may also be of major importance in this transformation.

It would appear then that the importance of the true iron bacteria has been overemphasized, whereas the importance of the heterotrophic bacteria in transformations of iron in nature has not been fully appreciated.

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THE USE OF HYDROGEN PEROXIDE FOR ESTIMATING HUMIFICATION

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The work described in this note was carried out some years ago, in order to estimate the value of the 6 per cent hydrogen peroxide method for determining "degree of humification" in soils or decomposed organic materials.   Clearly if a method is to effect even "an approximate separation between humified and unhumified matter" (4) the reagent used should have the minimum of action on unhumified material.   In the original description of the method (4) it was shown that cellulose and "crude fiber" from hay, straw, and wood were negligibly attacked, but that washed hay and straw suffered appreciable loss (17.8 and 11.5 per cent respectively).   Since in point of fact it is not "crude fiber" but whole plant material that is decomposed in the formation of humus, it was thought necessary to have more information concerning the extent to which non-humified plant materials are affected by the 6 per cent peroxide reagent before it was used for comparing the humification of materials of different origin.   The results were so discouraging to the adoption of the method for quantitative purposes that the work was carried no further.

Recently, however, in a critical study of methods for determining the "humified" portion of the soil organic matter, Waksman and Stevens (5) examined the 6 per cent peroxide method along with others in studying in considerable detail four materials —sound wood (finely ground), rotted wood, forest soil, and lowmoor peat soil.   After comparing the action of hydrogen peroxide and chlorine dioxide on certain fractions of these substances, those authors concluded that results with oxidizing agents could not be interpreted in terms of definite constituents of the plant residues, and so could not be used in determining degree of decomposition.   They further commented on the extent (20 per cent) to which even sound wood was oxidized by the peroxide reagent.   As an extension of this observation, it seemed useful to put on record the results of the present author's work: here the action of peroxide on a number of fresh plant materials had been examined, along with the extent to which it might be attributed to simple solvent action, while the further influence of soil on peroxide action was made clear.

It was found that the action of 6 per cent peroxide on all the plant materials examined was considerable, varying from 19 per cent with straw to 61 per cent with mustard tops; the effect was increased in every case by mixing with soil,

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with values then ranging from 21 per cent (straw) to 70 per cent (sphagnum). With most materials, however, a great part of the effect of the peroxide reagent was simply solvent action—that is to say, treatment with water alone under the conditions of the method caused considerable losses, and the extracted residues were more resistant to peroxide. With two exceptions (sphagnum, 49 per cent, and mustard, 31 per cent) none of the residues after water extraction lost more than 16 per cent of their weight under peroxide treatment.

This result might supply some justification for the use of the peroxide method —so far as its action on undecomposed material is concerned—if it were possible to assume that under natural conditions the water-soluble portions of plant materials were completely leached away by rain and played no part in "humification." But this is unlikely, and in any case the catalytic influence of the inorganic matter, in soils, would increase the action of peroxide on the insoluble plant material. Consequently, from the point of view of the action of the peroxide reagent on *undecomposed* plant material, estimates of "degree of humification" by this method can only be very approximate; and with some materials, particularly those including sphagnum, quite unreliable.

From the point of view of the action on *decomposed* plant material, the adverse verdict of Waksman and Stevens has already been noted. In a paper of which only the abstract is at present available McLean (3) examined the action of hydrogen peroxide in a different way, by studying the effect of varying concentrations on organic matter in the soil. Regular variations in the attack of peroxide on soil nitrogen and carbon were found indicating an optimum concentration of peroxide (3 per cent), at which soil organic matter was differentiated into "two categories whereby it is possible to arrive at the condition of the organic matter of a soil at any particular time." The relationship of these categories to the degree of humification of the organic matter does not seem to be sharply defined; in any case if the 3 per cent reagent were to be adopted for this determination it would seem desirable first to examine its action on a variety of undecomposed plant materials.

In discussing the use of 6 per cent peroxide for estimating degree of humification it should not be overlooked that in practice the method has given some degree of agreement with visual observations and the known history of samples (1, 2). It seems clear that decomposed substances are more attacked than fresh materials [compare also "fresh" and "rotted" wood (5)], whether the reason is largely a matter of changing chemical constitution or in part mechanical, a result of the disintegrated condition of the decomposed material. But quantitative significance cannot be attached to results obtained by the method.

EXPERIMENTAL

The procedure employed in the present work was based on that described by Glomme (1), working with forest soils.

Two grams of oven-dried material was treated in a 250-cc. beaker with 60 cc. of 6 per cent hydrogen peroxide, heated on a water bath for 15 minutes, brought

to the boiling point over a flame, and filtered while hot through a fluted filter paper. The residue was completely washed out of the beaker with hot peroxide, and further washed on the filter with boiling peroxide until visible reaction (production of bubbles) ceased. Then after being washed three or four times with boiling water, it was transferred to a tarred porcelain or silica dish, dried in an over for about 15 hours, weighed, ignited at red heat, and again weighed. In this way it was possible to estimate both "peroxide loss" and loss on ignition.

In the first series of experiments, samples of various plant materials were treated by this "standard method." In a parallel series, the materials were mixed with soil: 2 gm. of a mixture containing 20 per cent of added plant

TABLE 1

Action of hydrogen peroxide on unhumified plant materials

	PEROXIDE LOSS	PEROXIDE LOSS IN PRESENCE OF SOIL	SOLUBLE IN BOILING WATER	PEROXIDE LOSS AFTER EXTRACTION	TOTAL LOSS, BOILING WATER FOLLOWED BY PEROXIDE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Barley straw	16 8	21	11	8 [9]*	19
Hay	16 9	23	10	4 [5]	14
Larch needles	18 3	29	14	9 [10]	23
Beech leaves	21 6	27	15	8 [9]	23
Beech leaves, autumn	25 1	38	3	15 [15]	18
Pine needles	29 7	38	16	11 [13]	27
Lucerne tops	30 0	41	26	3 [4]	29
Oak leaves	30 5	38	21	5 [7]	26
Pasture grass	32 0	35	22	4 [5]	26
Oak leaves, autumn	34 8	44	13	14 [16]	27
Fungus mycelium	46 6	60	35	9 [14]	44
Sphagnum moss	48 3	70	5	46 [49]	51
Mangold leaves	48 3	67	41	5 [9]	46
Mustard tops	60 7	65	31	22 [31]	53

* Figures in brackets are expressed as per cent of insoluble organic material
To these values may be added those (already quoted) obtained by other investigators who examined undecomposed plant materials "peroxide loss" of straw, 11 5 per cent, hay 17 8 per cent; finely ground chestnut wood 20 1 per cent

material was used, and the loss in weight of the plant material was obtained by difference, the behavior of the soil alone under the standard method being first ascertained. In this series it was found that ordinary filter paper was weakened by hot peroxide in the presence of soil, and hardened paper (Whatman 50) was used. In another series, the "standard method" was followed except that distilled water was used throughout instead of hydrogen peroxide, the final volume of filtrate being brought to the same level as in the standard method. The residue, after being dried and weighed, was then treated with peroxide by the standard method.

The materials examined were taken as typical of those which play a consider-

able part in the production of humus in different kinds of soil; for forest soils, were chosen pine and larch needles, and the leaves of oak and beech (both green leaves and recently fallen autumn leaves); for arable soils, barley straw, mangold leaves, and lucerne and mustard tops; for grassland, hay and young pasture grass were taken. Sphagnum moss was also examined, as being a major component of many peats; and the stems of the fructifications of a fungus, *Lepiota procera*, whose mycelium represents another material that contributes to "humus" formation. The samples were dried in a warm room, roughly ground in a hand mill, sieved (3 mm.), and oven dried for 20 hours. The soil used in one series of experiments was a Rothamsted (clay loam) soil poor in organic matter; it was similarly sieved and dried.

The results obtained are summarized in table 1. They are expressed as per cent of organic matter, taken as equal (for the purpose of this method) to the loss on ignition. The column "peroxide loss" expresses the loss in weight through treatment with 6 per cent hydrogen peroxide, which has been called the "degree of humification" in studies of decomposed materials. The values in this column are means of two or more determinations, which generally agreed closely; the greatest difference was under 2 per cent.

It is tempting to discuss these results in detail, especially in relation to what is known of the chemical composition of the different materials. High nitrogen substances after water extraction, for example, are particularly resistant, a result which may be compared with McLean's finding (3) of a resistant form of nitrogen in the soil organic matter. The measurements, however, are highly empirical; in addition to minor sources of inaccuracy, the results would be affected by changes in factors such as fineness of grinding or stage of growth of the material used. Their purpose was simple to supply information bearing on the use of the "6 per cent peroxide method," and from this point of view the results have been reviewed in the introduction.

SUMMARY

A number of undecomposed plant materials have been treated by the 6 per cent hydrogen peroxide method as used for estimating "degree of humification."

The action of the peroxide on these materials was far from negligible and was still greater in the presence of soil.

With the majority of the materials examined, simple solvent action was responsible for most of the loss under peroxide treatment, although there were notable exceptions.

From the point of view of its action on undecomposed materials the 6 per cent peroxide method can give only approximate results, and it is inadvisable to use it for comparing materials of widely differing origin.

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THE ALCOHOL METHOD FOR DETERMINING MOISTURE CONTENT OF SOILS

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In previous preliminary communications (1, 2) the alcohol method was proposed as a rapid and accurate method for determining the moisture content of soils. It was shown that by means of methyl alcohol the moisture content of soils could be determined almost as accurately as by the drying oven method, and the time required for such a determination was short, ranging from about 5 minutes in the case of sandy soils to about 12 minutes for the heaviest clays. It was strongly emphasized that the accuracy and success of the method depends upon the alcohol coming into intimate contact with the entire soil mass and in order that this condition may be attained it is essential that the soil be thoroughly dispersed. To quote, "The soil, however, should always be reduced to the particle state so the alcohol can come in contact with the soil mass."

Smith and Flint (3) undertook to determine the accuracy of the method on a large number and variety of California soils, using the drying oven method as a standard for comparison. The results they obtained go to show that in the majority of soils, especially in the clays, the alcohol method was really a failure in determining the moisture content with any degree of accuracy.

Apparently their inability to obtain satisfactory results was due to a failure to effect complete dispersion of the soil mass. As stated in the foregoing, the first prerequisite for the accuracy and success of the method is a complete dispersion of the soil so its entire mass will come into intimate contact with the alcohol. But Smith and Flint apparently did not obtain a complete dispersion of the soils.

In dispersing the soils the writer originally used an iron rod about a quarter of an inch in diameter and flattened somewhat at the end, and by means of which it was found possible to disperse rather completely almost every kind of clay in probably not more than 10 minutes.

It is very obvious, however, that in trying to disperse the soils by hand with such a rod, the personal element enters, which makes uncertain and indefinite the degree of dispersion that the soils receive under different operators; consequently it has seemed advisable to devise a mechanical means of dispersing the soils. For this purpose, the idea was hit upon of using the milk shaking

machine such as is employed in the hydrometer method for mechanical analysis. Several modifications had to be made, however, before this mechanical means of dispersion was efficient and practical. In the first place, a special arrangement had to be made so the alcohol would not evaporate during dispersion. For this purpose, a narrow-necked-pint bottle was selected. A cork was fitted and tied at the top of the stirring rod. This cork was smoothed down so it would fit the neck of the bottle very tightly. Then on the paddle of the stirring rod was tied by means of wire, or soldered, a small steel spring such as those used with oil wicks on electric motors. This spring proved effective as an aid to dispersion. It would break up a ball of clay almost instantly, whereas without this spring the ball of soil would be stirred around and a long time would elapse before it was broken up or dispersed. The spring really adds enormously to the efficiency of dispersion of the outfit. The spring, however, should not be too long and rub on the bottle. This complete dispersion outfit is shown in plate 1.

The technique that was finally adopted for determining the moisture content of soils is as follows:

First, the hydrometer is calibrated. This is accomplished as follows: The specific gravity of pure methyl alcohol which is to be used, is first ascertained. For purpose of example, let it be assumed that it is 99.85 per cent alcohol. Exactly 75 cc. of this stock alcohol is carefully measured in a 100-cc. cylinder, 10 cc. of distilled water added to it, and the specific gravity again determined. Suppose this indicated 91.10 per cent alcohol in the mixture. The temperature must be recorded and the readings reduced to the same basis, namely 60°F. A temperature of 1°F, makes a difference of about 0.15 per cent alcohol. For temperatures above 60°F. the corresponding amount is subtracted from the percentage of alcohol indicated, and for temperatures below 60°F. the corresponding amount is added to the percentage of alcohol indicated.

When the readings are reduced to the same basis, then the reading of the alcohol which contained the 10 cc. of water is subtracted from the reading of the pure alcohol and the difference is divided into the 10 cc. of water. This gives the number of cubic centimeters of water to which each degree on the stem is equivalent. In a mixture of 75 cc. of 99.85 per cent alcohol and 10 cc. of water the standard special hydrometer gives 0.910 cc. of water for each graduation. To find the number of cubic centimeters of water in the soil sample taken the difference in specific gravity of the filtrate and pure stock alcohol is multiplied by 0.910. If 5 cc. of water is used with 75 cc. alcohol, the factor is a little less than 0.910.

Second, the general procedure for executing a moisture determination is as follows: About 25 gm. of moist soil are weighed out accurately and placed in the bottle. Exactly 75 cc. of pure methyl alcohol is poured into the bottle, the latter connected to the stirring machine and the contents stirred for about 2 minutes. In case of some soils which are seen not to have been dispersed in 2 minutes, they should be stirred a little longer. The contents of the bottle

are then poured into a filter paper on a 5-inch funnel and the filtrate allowed to drop into a 25-cc. regular graduated glass cylinder. The very first filtrate is usually turbid and consequently it is poured back into the funnel. It is well to repeat the latter process twice because it insures a clear filtrate and also serves to rinse thoroughly the graduated cylinder. Immediately after the alcohol-soil mixture is poured into the funnel, the latter is covered with a cover glass or some other means in order to prevent evaporation of the alcohol. This precaution is essential. When about 20 cc. of the filtrate is drained into the cylinder the funnel is removed and a special small alcohol hydrometer[2] is placed in the filtrate and its specific gravity and temperature are measured. From all the data at hand, the moisture content of the soil is then calculated.

EXPERIMENTAL RESULTS

In order to establish the principle with certainty that alcohol can take out all the water from a soil it is essential that the experiments be conducted under the most ridigly controlled conditions. For this purpose it was chosen to make the real test on oven-dry soils to which was added a definite and known quantity of water and then try to recover by the alcohol method the water added. This would seem to be a more logical and reliable procedure for establishing the principle, then trying to use natural moist soil, since it is difficult, and in many cases almost impossible, to take absolutely duplicate samples of natural moist soils. When the results under controlled conditions are definitely known the results obtained on natural soils can be more intelligently interpreted. The results presented in table 1, therefore, were obtained on dry soils that had passed through the 2-mm. mesh sieve. About 25 gm. of each soil was placed in the dispersion bottle, dried in an electric oven for 24 hours at a temperature of 110°C., and then placed in desiccator over sulfuric acid. When the soil had cooled 10 cc. of water was quickly added to it, and the bottle was stoppered to prevent evaporation. After sufficient time had elapsed for the soil to absorb the water the bottle was opened and 75 cc. of the stock alcohol was added. The bottle was then attached to the stirring motor and the soil mixture stirred for about 2 minutes. The alcohol mixture was then filtered, the first filtrate always being poured back, and the specific gravity determined. From the data obtained, the amount of water recovered by the alcohol was computed and compared to that added. Table 1 shows the type of results obtained from a comprehensive number of soils and artificial materials.

The results in table 1 show that the water added to the oven-dry soils is all or nearly all recovered by the alcohol. It will be seen that the amount that was not recovered is small in all cases.

These results, involving soils of maximum extremes in texture, go to establish

[2] These special alcohol hydrometers are made by Eimer and Amend. They come in pairs, one has a range of from 90 to 100 per cent alcohol and the other from 80 to 90 per cent. They are graduated into 0.5 and have a thermometer attached to them.

the principle, therefore, that the alcohol can take out practically all the water from the soil. The conclusion would seem to be justifiable, therefore, that the alcohol can be employed as a means of determining rapidly and accurately the moisture content of soils.

When the principle is definitely established that the alcohol can take out practically all the water from the soil, then the accuracy of the alcohol method for determining the moisture content of soils would depend upon the question of technique.

One of the most uncertain and difficult phases of the technique is to obtain a complete dispersion of the soil. If the soils are reduced to the particle state so the alcohol can come into contact with the entire soil mass the results in table 1 show that there is no question as to the accuracy and reliability of the method. If, on the other hand, the soils are not completely dispersed then

TABLE 1

Percentage of water recovered by methyl alcohol from water added to oven-dry soils

SOILS	AMOUNT OF WATER RECOVERED	SOILS	AMOUNT OF WATER RECOVERED
	per cent		*per cent*
Quartz sand.	100 50	Parson silt loam B.	99 94
Plainfield sand.	100 40	Sharkley clay.	100 04
Fine sand.	100 50	Lake Charles clay.	99 90
Miles sandy loam	100 30	Fargo clay.	99 89
Kirvin fine sandy loam.	99 95	Susquehanna clay.	99 10
Colby silt loam .	100 10	Capay clay.	100 05
Grundy silt loam	99 93	Sacramento clay.	99 . 40
Ontonagon clay B.	99 95	Bentonite.	99 19
Welland clay (Ontario)	99 85	Silica gel.	99 40
Haldimond clay (Ontario) . .	99 91	Muck.	99 15
Osage clay.	100 05		

the results will be very inaccurate and unreliable. It seems, however, that the new device for dispersing the soils, as already described, insures complete dispersion of practically all soils. This point was thoroughly tested by using a large number of some of the heaviest clays under different moisture contents and structural condition. Some of these clays include Susquehanna clay, Capay clay, Osage clay, Sharkley clay, Welland clay, Haldemand clay, Lake Charles clay, and Fargo clay. These clays were either used in the natural moist condition or worked into paste and then into balls of different moisture contents. They were then subjected to dispersion by the stirring machine and at the end of 2 or 3 minutes they were examined to ascertain the degree of dispersion that they had undergone. It was found that in practically every case they had been reduced to the particle size.

If these clays can be completely dispersed by the stirring machine then the natural and logical conclusion from the results in table 1, is that their moisture

content can be accurately determined by the alcohol. Although this appears to be undoubtedly the case, yet, on account of the great difficulty of obtaining duplicate samples that are exactly alike in moisture content, the comparison of the moisture determination between the alcohol and drying oven methods, may not always be very close or very certain. In table 2 is shown the comparison of the moisture content, as determined by the alcohol and drying oven method, of a number of the clays. Some of these clays[3] were used in their natural moist condition; some of the other clays were worked by hand into balls of different moisture content.

The results in table 2 go to show that where the soils were worked by hand so the moisture content would be more uniformly distributed and thereby

TABLE 2

A comparison of moisture content, as determined by the alcohol and drying oven methods, of clays with natural moisture content or worked into balls by hand

SOILS	MOISTURE DETERMINED BY		REMARKS
	Alcohol	Drying oven	
	per cent	*per cent*	
Sharkey clay	33 38	34 50	Natural moist soil
Miami clay	20 46	21 96	Natural moist soil
Miami clay	25 95	25 14	Natural moist soil
Miami clay	18 45	19 64	Natural moist soil
Grundy silt loam	40 80	41 40	Worked into hard ball
Vence	22 16	22 88	Worked into ball
Susquehanna clay	27 84	28 16	Worked into ball
Asage clay	31 18	31 97	Worked into ball
Ontonagon clay	28 69	28 53	Worked into ball
Welland clay	33 05	32 85	Worked into ball
Fargo clay	35 16	36 05	Worked into ball
Osage clay	43 70	42 83	Worked into ball
Osage clay	40 20	40 55	Worked into ball

afford more uniform duplicates, the moisture content as determined by the alcohol and drying oven methods agree very closely, whereas in the case of the natural soils where uniform duplicates are difficult to obtain the agreement is not as close.

It seems almost incredible that the alcohol can reduce the moisture content of soils to almost oven-dry basis (tables 1 and 2). It is possible that the alcohol does not take out all the last traces of water in the soils, but that any traces of water that it fails to extract or replace are probably compensated by traces of material that the alcohol may dissolve out. This hypothesis would seem to be supported by the fact that in very heavy alkali soils the alcohol-water mixture dissolves the salts, and consequently the alcohol method cannot be

[3] Kindly furnished by Dr. C F. Marbut.

used unless a correction is applied. On the other hand, in mediumly alkali soils, such as those occuring in humid regions, the method seems to work just as accurately as in the non-alkali soils. This point was tested by trying the method on certain alkali soils occurring in Iowa and the results obtained were similar to those shown in table 1.

From all the studies thus far made, there seems to be no doubt that the alcohol method, when properly used, can determine the moisture content of soils almost as accurately as the drying oven. On the other hand, to achieve this accuracy more careful technique is required in the alcohol method than in the oven-dry method.

It must be emphasized that the alcohol method for determining the moisture content of soils is not offered with the intention of replacing the oven-drying method, especially for scientific purposes. The value of the alcohol method lies in showing that the alcohol is able to reduce the moisture content to almost the oven-dry basis, in affording a method for special cases where a very rapid method is desired, and in affording a rapid and practical method for situations where other methods cannot be so easily used, such as out in the field and in field stations.

SUMMARY

The alcohol method for determining moisture content of soils very rapidly has been reinvestigated. The results obtained go to show that if the method is properly used it is accurate and reliable.

The method can determine the moisture content of soils in about 5 minutes in the case of light textured soils, and in about 12 minutes in the heaviest clays.

A new feature is introduced in the method, whereby the soils are dispersed mechanically by means of the milk shaking machine. By tying a spring to the paddle of the stirring rod, even the heaviest clay can be reduced to the particle state in a few minutes.

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PLATE 1

SOIL DISPERSING MACHINE SHOWING THE SPRING, CORK STOPPER, AND BOTTLE
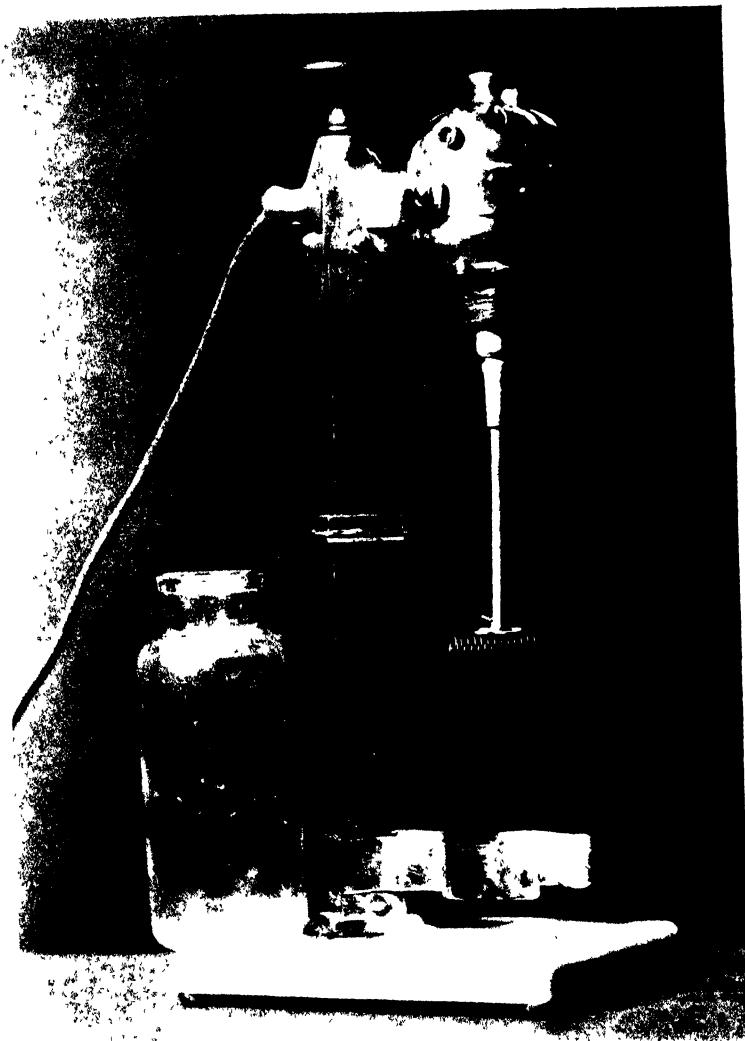
THE MOISTURE EQUIVALENT AS A MEASURE OF THE FIELD CAPACITY OF SOILS

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The moisture equivalent of Briggs and McLane (5) with the modification of the method later made by Briggs and Shantz (6) is used widely by American investigators in interpreting soil-moisture conditions. We believe that the moisture equivalent is a fairly reliable measure of the texture of the soil. A number of formulas have been suggested to indicate the relation of the moisture equivalent to the mechanical analysis (2, 6, 14, 17). All of these differ and, therefore, seem to cast doubt on the reliability of the moisture equivalent as a measure of soil texture. Since the moisture equivalent is determined by a purely arbitrary method, the differences in the formulas may be due, at least in part, to lack of uniformity in the technique of making the determinations. We have found, however, that the moisture equivalent made on 30-gm. samples according to the method previously described (23, 24) may be used as a satisfactory measure of the relative texture of soils and may, also, be used as a measure of the relative wetness of usual agricultural soils except sands (8).

Many investigators have reported tests which indicate that the moisture equivalent is a close approximation of the amount of water that the soil in the field will retain against the pull of gravity. This moisture condition of field soils is referred to in a number of ways, among which are: specific retention, maximum water-holding capacity, field-carrying capacity, normal field capacity, and maximum field or capillary capacity. We prefer the use of the term *field capacity* to indicate the amount of water held in the soil after the excess gravitational water has drained away and after the rate of downward movement of water has materially decreased, which usually takes place within 2 or 3 days in pervious soils of uniform structure and texture. As Israelsen (12) has pointed out, soils may drain slowly for a long time after irrigation, but in all of our experiments the rate of extraction of the water by plants was greater than this downward movement.

In any event, the need for a clear definition of the term used is apparent when the confusion of meaning is found in some of the reports on soil-moisture studies. In many cases the moisture contents are reported simply as certain percentages of saturation, leaving the reader in doubt as to whether complete filling of the pore space of the soil, or field capacity in the sense defined in the foregoing, is meant by saturation. In others, the soil-moisture contents are reported on the basis of the moist weight of the soil, or the percentages of moisture are given on the volume basis instead of that of the weight of the soil.

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RESULTS OBTAINED BY OTHER INVESTIGATORS ON THE RELATION OF MOISTURE
EQUIVALENT TO FIELD CAPACITY

In tests on loessial soils with moisture equivalents above 21 per cent Burr and Russell (7) found the average field capacity for the upper 6 feet of soil to be 91 per cent of the average moisture equivalent. Israelsen's (11) results with loams and clays which were heavily irrigated and which have moisture equivalents greater than 19 per cent, show that the maximum quantity of water held after irrigation was slightly smaller than the moisture equivalent, being about 90 per cent. Scofield and Wright (15), working with a fine sandy loam soil with moisture equivalents from about 13 to 17 per cent, concluded "that the moisture equivalent of this soil affords a fair basis for estimating its field carrying capacity after the conditions of equilibrium have become established." Mathews (13) found from tests at 17 stations in the Great Plains that the field carrying capacity of a soil is a little lower than the moisture equivalent, but bears a linear relation to it. Harding (9) has summarized the results of some 10,000 soil-moisture samples taken before and after irrigation of field plots for the purpose of comparing the moisture equivalent with the critical moisture contents of soils under field conditions of irrigation practice. He concludes "that the relationship of the soil-moisture properties to the moisture equivalent does not appear to be linear except in the case of the wilting of the crop." Harding had only 136 moisture equivalents ranging from 4.1 to 37.6 of the large number of soil samples he considered. As pointed out elsewhere (8, p. 116) serious error may be introduced if moisture equivalents are made on but a few of the samples taken. Harding's data indicate that the field capacities of the sands were higher than the moisture equivalents, whereas those of the finer texture soils were lower than the moisture equivalents.

Alway and McDole (1) from laboratory and field experiments also found the water-retaining capacities of the sands to be higher than the moisture equivalents, but those of the loams were closer to the moisture equivalents. Shaw (16) concluded from laboratory experiments that the "normal field capacity" for medium textured soils was approximately the same as the moisture equivalent.

RELATION OF MOISTURE EQUIVALENT TO PERMANENT WILTING PERCENTAGE

We have found (10, 22) that the residual moisture content of the soil at the time plants permanently wilt does not bear a linear relation to the moisture equivalent, and our results show that plants are able to reduce the moisture content of different soils to different degrees of dryness, as measured by the moisture equivalent. Furthermore, the ratios of the moisture equivalents to moisture contents at permanent wilting bear no relation to the type of soil, high and low ratios being found with sands, with loams, and with clays. The question then arises whether the moisture equivalent is a measure of the relative moisture-retaining properties of soil. Obviously, the moisture equivalent can not measure soil structure, since the original structure of the soil may be

changed greatly in preparing the samples for centrifuging and in the centrifuging process itself. We have, therefore, attempted to measure the field capacities of some of the soils we used in our experiments on the wilting of plants and have compared them with the moisture equivalents. Moisture equivalents were made as previously described (23, 24), but with better speed control of

TABLE 1

Field capacities, moisture equivalents, and relative wetness* of some agricultural soils in California

The field capacities and moisture equivalents are given as percentages on the basis of the dry weight of soil.

SOIL	LOCATION	FIELD CAPACITY	MOISTURE EQUIVALFNT, AIR DRIED SAMPLES	RELATIVE WETNESS	
				Air dried samples, March, 1930	Oven-dried samples August, 1930
Tehama loam	Arbuckle	15 2 ±0 13	14 1 ±0 07	1 08	1 16
San Joaquin loam	Wheatland	19 2 ±0 35	17 9 ±0 07	1 07	1 10
Columbia sand	Sheridan	21 5 ±0 45	17 9 ±0 30	1 20	1 31
Columbia silt loam	Sacramento	19 5 ±0 32	17 2 ±0 51	1 13	1 26
Columbia silt loam, 1st foot	Colusa	22 9 ±0 14	19 6 ±0 11	1 17	1 23
Columbia silt loam, 2nd foot	Colusa	16 7 ±0 33	14 2 ±0 47	1 17	1 26
Columbia silt loam, 3rd foot	Colusa	18 8 ±0 58	16 5 ±0 16	1 14	1 30
Columbia silt loam, 4th foot	Colusa	26 6 ±0 72	25 9 ±0 28	1 03	1 15
Columbia silt loam, 5th foot	Colusa	26 3 ±0 49	26 8 ±0 25	0 98	0 96
Sacramento silt loam, 1st foot	Los Molinos	25 0 ±0 33	20 0 ±0 12	1 25	1 34
Sacramento silt loam, 6th foot	Los Molinos	24 3 ±0 29	23 3 ±0 35	1 04	1 11
Stockton clay adobe	Pleasant Grove	21 2 ±0 36	22 1 ±0 21	0 96	0 99
Tehama clay	Zamora	21 4 ±0 40	20 4 ±0 34	1 05	1 09
Farwell silt loam	Chico	23 5 ±0 09	22 9 ±0 03	1 03	1 07
Capay clay	Plainfield	25 5 ±0 17	25 7 ±0 10	0 99	1 04
Aiken loam, 1st foot	Camino	25 4 ±0 47	26 6 ±0 46	0 95	0 98
Aiken loam, 2nd foot	Camino	25 5 ±0 31	26 4 ±0 13	0 96	1 04
Aiken loam, 3rd foot	Camino	25 5 ±0 16	26 3 ±0 19	0 97	1 02
Aiken loam, 4th foot	Camino	25 3 ±0 28	25 9 ±0 35	0 98	1 04

* Relative wetness is the ratio of the field capacity to the moisture equivalent expressed decimally

the centrifuge than was found possible with the governor supplied with the standard moisture equivalent centrifuge (19).

RELATION BETWEEN MOISTURE EQUIVALENT AND FIELD CAPACITY

In March, 1930, samples were taken in fields from which the original supply of some of the soils were taken for our studies on the wilting of plants. The location of the first place of sampling was not accurately known in every case,

hence moisture equivalents of these soils listed in the following tables do not agree in every case with the moisture equivalent of our original samples.

The samples listed in table 1 were taken close to the end of the rainy season after a rainy period that was followed by 2 days of clear weather just previous to the time the samples were taken. The surface soil to a depth of about 5 inches was removed and the samples taken from about the 5 to 10-inch depth. In some cases samples were taken to a depth of 6 feet. With the exception of the Aiken loam at Camino, all of the samples were taken from cultivated fields. Undoubtedly the compacted layer below the depth of tillage in some cases had prevented complete drainage taking place during the 2 days following the rain. The samples of Columbia sand at Sheridan and the first foot of Sacramento silt loam at Los Molinos showed some free water when taken. The Columbia silt loam at Colusa was irrigated just before the samples were taken, which probably is the reason for the high relative wetness in the upper layers of this soil. Therefore, determinations of the field capacities of the soil given in table 1, are not as accurate as those of the soils listed in table 2, which were obtained under much more carefully controlled conditions.

The samples were divided, one portion being used for the determination of the moisture content by drying for 48 hours at 110°C. and the other was air dried. Moisture equivalents were determined on both the air-dried and oven-dried samples. Six months later moisture equivalents were again run on the oven-dried samples, which had been stored in paper sacks in the laboratory. The results are listed in table 1. It will be noted that oven drying changes the moisture equivalents of some soils but not of others, which is in accord with previous observations (24). In only one instance was the moisture equivalent of the oven-dried samples higher than that of the air-dried samples. It is clear that storage of the samples for 6 months did not restore the soil to a condition which gave moisture equivalents the same as those of the air-dried samples. The average ratio of the field capacity to the moisture equivalent of the air-dried samples is 1.06 ±0.023, whereas the ratio for the oven-dried samples in March is 1.11 ±0.026, and for those in August, 1.13 ±0.026. It is obvious, therefore, that the method of preparing the samples may affect the results of the comparisons between field capacity and moisture equivalent. Oven-drying increased the difference between the field capacity and moisture equivalent in the majority of the tests listed in table 1. In some of our later tests it was not possible to use air-dried samples, so both air-dried and oven-dried samples are included in the table to show the discrepancies that are sometimes due to this factor. We feel that the best comparison between field capacity and moisture equivalent can be made on air-dried samples. Furthermore, the uses of different quantities of soil in the moisture equivalent centrifuge cups and differences in methods of preparing the samples may lead to further discrepancies.

The data in table 1 indicate that moisture equivalents of more than 14 per cent may be used as a fair measure of the field capacities of these soils.

Where compacted layers are formed by tillage, penetration of the water applied may be slowed up to such an extent that more than 2 days must be allowed for drainage before samples are taken for field capacity determination. Burr and Russell (7), Mathews (13), and Israelsen (11) all report the field capacity of their soils to be a little lower than the average moisture equivalent.

An instance of the consistency encountered in making field capacity tests at Davis is illustrated by the following data. After a heavy rain, samples were taken throughout the portion of the soil which was wetted, the depth of the sampling ranging from 2 to 16 inches. The average moisture content was 22.7 per cent and the average moisture equivalent was 22.2 per cent, a ratio of field capacity to moisture equivalent of 1.02. Several years later another test was made in another portion of the same field from which the foregoing data were taken, and the ratio of field capacity to moisture equivalent was found to be 0.97. The plot was covered to prevent evaporation and the entrance of rain, and was left for about 2 months, and a second set of samples was obtained. The ratio again was found to be 0.97. We have shown (20, 22) that the field capacities of the loam soils at Davis are very close to the moisture equivalents.

A slightly different type of test from those just described to determine the field capacities and their relation to the moisture equivalents was employed in the fall of 1928. Plots on Yolo clay at Davis; Madera and Gridley loam near Yuba City; Fresno sandy loam near Hughson; Hanford fine sandy loam near Riverside; and Oakley fine sand at Delhi were selected. Attempts were made to obtain plots on dry soil far enough removed from growing plants to prevent losses by transpiration, but it was not possible to get dry soil at all places. The plots were square, 8 feet along each side, and were enclosed by a wooden frame. There were two plots in each test with a space of about 2 feet between them. The wooden frame was set level and backed up with soil so water could not seep away from the plots. The soil was levelled by scraping off the high spots. Eight samples, evenly spaced, were taken around the outside of each plot to ascertain the moisture content of the soil before applying water to the plots. Water was applied to the plots through a meter so that the depth of application was known rather accurately. The plots were covered with canvas to prevent loss of evaporation and several sets of samples were taken within the plots. Eight samples, evenly spaced, from each plot comprised a set. These were dried for moisture determinations and the moisture equivalent determinations were made later on the oven-dried samples. The samples were taken with the soil tube previously described (18) and which has been shown can be used for volume weight determinations of the soil (3). Since it was necessary to find the volume weight of the soil we did not wish to introduce errors into this determination, as well as into that of the moisture content, by subdividing the samples to obtain a portion for air drying for the moisture equivalents. The results of the tests are given in table 2.

A trench was dug across the plots in three of the experiments after the last

TABLE 2

The relative wetness* of five California soils before and after irrigation

LOCATION	SOIL	DEPTH OF WATER APPLIED	TIME SAMPLED	RELATIVE WETNESS OF SOIL Depth in inches			
				0–12	12–24	24–36	36–48
		inches					
Davis	Yolo clay	8	Before irrigation	0 47	0 66	0 79	0 88
			3 days after	0 85	0 81	0 97	0 98
			6 days after	0 92	0 82	0 97	1 00
			9 days after	0 88	0 78	0 97	0 97
		12	Before irrigation	0 47	0 65	0 82	0 92
			3 days after	0 95	0 94	1 02	1 08
			6 days after	1 01	0 94	1 08	1 10
			9 days after	0 97	0 91	1 01	1 06
Yuba City	Madera and Gridley loam	4	Before irrigation	0 22	0 41	0 50	0 53
			2 days after	1 06	0 73	0 52	
			3 days after	0 97	0 64	0 51	
			4 days after	0 95	0 64	0 49	
		8	Before irrigation	0 22	0 37	0 49	0 52
			2 days after	1 06	0 93	0 76	0 64
			3 days after	1 02	0 94	0 83	0 63
			4 days after	0 99	0 85	0 78	0 65
Hughson	Fresno sandy loam	4	Before irrigation	0 31	0 31	0 34	0 39
			2 days after	1 42†	0 68	0 52	0 33
			6 days after	1 11	0 71	0 39	0 37
		6	Before irrigation	0 41	0 45	0 54	0 61
			2 days after	1 30†	1 20	0 57	0 61
			6 days after	1 18	1 03	0 76	0 62
Riverside	Hanford fine sandy loam	3	Before irrigation	0 50	0 55	0 60	0 59
			2 days after	1 38	0 88	0 54	0 59
			4 days after	1 21	1 03	0 65	0 62
			5 days after	1 13	0 99	0 77	0 73
		6	Before irrigation	0 38	0 48	0 53	0 56
			2 days after	1 34	1 26	1 09	1 09
			4 days after	1 21	1 15	1 04	1 03
			5 days after	1 24	1 16	1 05	1 11
Delhi	Oakley fine sand	3	Before irrigation	0 74	0 86	0 96	0 96
			2 days after	1 72	1 47	1 15	0 96
			3 days after	1 67	1 48	1 28	0 96
			6 days after	1 38	1 38	1 32	0 96
		6	Before irrigation	0 78	0 88	0 90	0 81
			2 days after	1 93	1.99	2.06	1 26
			3 days after	1 54	1 64	1.88	1 39
			6 days after	1 54	1 38	1 54	1 26

* Relative wetness is ratio of moisture content to moisture equivalent expressed decimally.
† Surface soil was saturated.

set of samples was taken. The line of demarcation between the wet soil and the dry soil was mapped and samples were then taken at points within and without the wetted areas. The results are indicated in figures 1 to 6. The position of the small circles indicate the place of sampling and the numbers are the ratios of the moisture contents to the moisture equivalents made on the oven-dried samples. They are the relative wetness of the soil (8) but are given as decimals and not as percentages. These data, together with those in table 2,
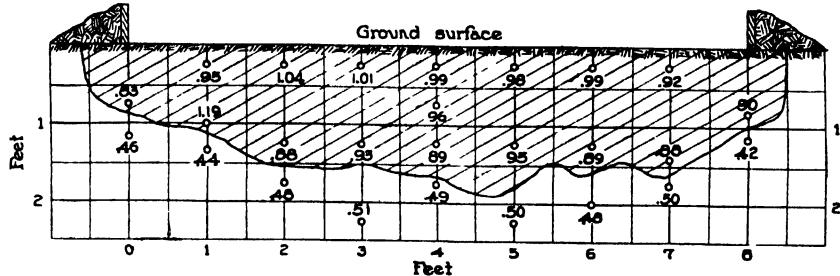

FIG. 1. FILLD CAPACITY PLOT AT YUBA CITY ON MADERA AND GRIDLEY LOAM 4 DAYS AFTER A DEPTH OF 4 INCHES OF WATER WAS APPLIED

The hatched area represents the wetted portion of the soil and the numbers are the relative wetness, or the ratio of moisture content to the moisture equivalent.
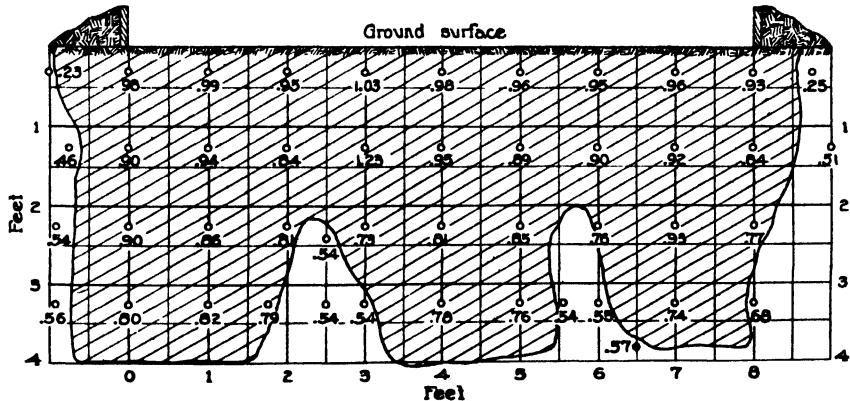
FIG. 2. FIELD CAPACITY PLOT AT YUBA CITY ON MADERA AND GRIDLEY LOAM, 4 DAYS AFTER A DEPTH OF 8 INCHFS OF WATER WAS APPLIED

The hatched area represents the wetted portion of the soil and the numbers are the relative wetness, or the ratio of moisture content to the moisture equivalent

show that the moisture equivalent of the Yolo clay and Madera and Gridley loam is close to the field capacity, but the moisture equivalents of the sandy soils, Oakley sand, Fresno sandy loam, and Hanford fine sandy loam are less than the field capacities. The relation of the field capacities to the moisture equivalent of some of these soils as shown by regular sampling in orchard plots throughout the growing season has been discussed in a previous publication (10).

Our results showing the relation between the moisture equivalent and the field capacity agree with the findings of Alway and McDole (1) and with those of Harding (9) regarding sandy soils, but not with his results on the finer textured soils. Beckett, Blaney, and Taylor (4) report the field capacities of their soils based on a large number of soil-moisture samples and a limited number of moisture equivalents which make the comparison less exact than in the case where a moisture equivalent is made on every sample. Their results
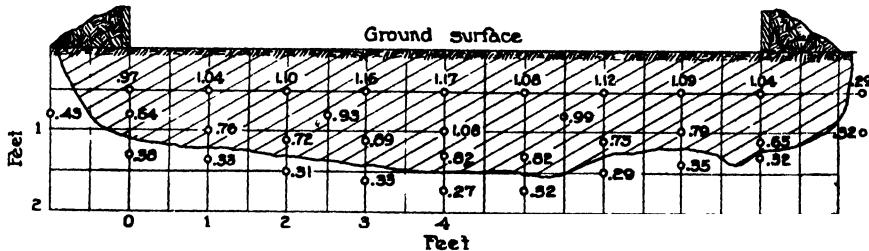

FIG. 3   FIELD CAPACITY PLOT AT HUGHSON ON FRESNO SANDY LOAM, 6 DAYS AFTER A DEPTH
OF 4 INCHES OF WATER WAS APPLIED

The hatched area represents the wetted portion of the soil and the numbers are the relative wetness, or the ratio of moisture content to the moisture equivalent.
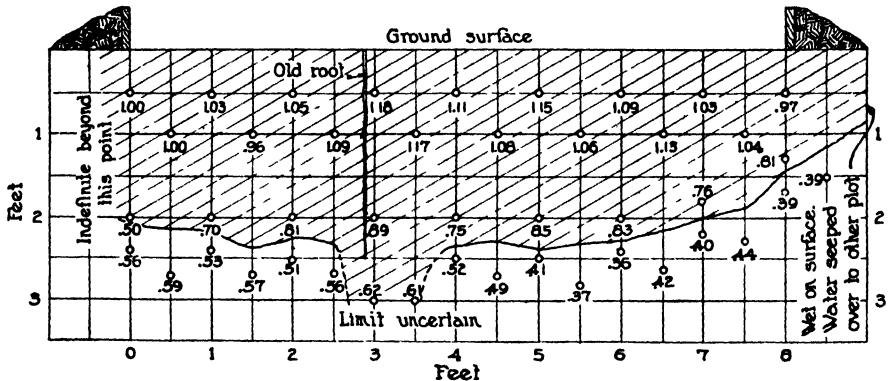

FIG. 4.   FIELD CAPACITY PLOT AT HUGHSON ON FRESNO SANDY LOAM, 6 DAYS AFTER A DEPTH
OF 6 INCHES OF WATER WAS APPLIED

The hatched area represents the wetted portion of the soil and the numbers are the relative wetness, or the ratio of moisture content to the moisture equivalent.

in three different orchards on Sierra sandy loam were as follows: moisture equivalent 9.2, field capacity 11.3; moisture equivalent 11.0, field capacity 14.0; moisture equivalent 13.7, field capacity 13.5 to 15.0. The plots on Sierra loam had a moisture equivalent of 9.9 and field capacity of 12.0. The Holland sandy loam had a moisture equivalent of 14.7 and field capacity of 14.1   Here again the field capacities of the sandy soil are higher than the moisture equivalents, whereas in the fine textured soils the field capacities and the moisture equivalents are approximately equal.

INFLUENCE OF SOIL STRUCTURE ON THE RELATION OF THE MOISTURE EQUIVA-
LENT TO THE FIELD CAPACITY

Among the factors that may influence the field capacity, and hence, the
relationship between the moisture equivalent and the field capacity of a soil
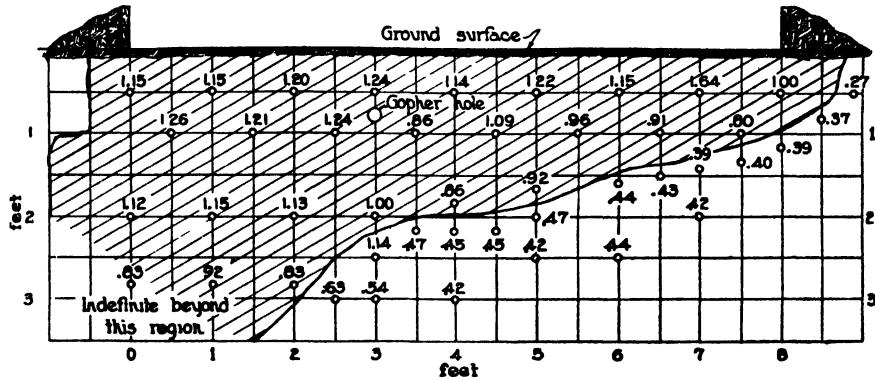
FIG 5 FIELD CAPACITY PLOT AT RIVERSIDE ON HANFORD FINE SANDY LOAM 5 DAYS AFTER A
DEPTH OF 3 INCHES OF WATER WAS APPLIED

The hatched area represents the wetted portion of the soil and the numbers are the relative
wetness, or the ratio of moisture content to the moisture equivalent.

FIG. 6 FIELD CAPACITY PLOT AT RIVERSIDE ON HANFORD FINE SANDY LOAM 5 DAYS AFTER
A DEPTH OF 6 INCHES OF WATER WAS APPLIED

The hatched area represents the wetted portion of the soil and the numbers are the relative
wetness, or the ratio of moisture content to the moisture equivalent.

are: soil texture, depth of soil column, depth to water table, temperature,
amount and kind of dissolved material in the soil water, and structure of the
soil. Alway and McDole (1) have shown that continuity of texture is essen-
tial for approximately uniform distribution of water in the soil. Our experi-

ments were concerned only with discontinuities in soil structure. They showed that discontinuities in structure have such a decided effect on the field capacity that the moisture equivalent often may not be taken as a measure of the field capacity.

The Madera and Gridley loams at Yuba City, the Fresno sandy loam at Hughson, and the Oakley fine sand at Delhi were underlaid at a depth of between 4 to 6 feet with compacted layers of soil through which water permeated very slowly. Therefore, there was the possibility of water accumulating above the impervious layer. We believe this did not happen, except possibly in the case of the 6-inch application of water on the Oakley fine sand which may have resulted in a penetration of water to this layer, and downward movement may have been intercepted, but even this is questionable.

The effect of soil structure on the field capacity is shown in figures 1 to 6. The plots on Hanford fine sandy loam (figs. 5 and 6) were about 30 feet from the outer row of trees in an orange grove and close to a road. The tillage implements were turned in this space. The right hand side of figures 5 and 6 were toward the orchard. The compacting of the soil just below the depth of tillage was greater on this side than on the other. Consequently the water moved downward more rapidly on the left than on the right side, as is indicated in the figures. The volume weight or apparent specific gravity of the Hanford fine sandy loam from 0 to 12 inches deep was found to be 1.46, while the next lower 2 feet were each 1.34, which indicates that the surface soil was compacted. Volume-weight determinations were made on samples taken in the orange grove adjacent to the plots. The samples taken under the trees 2 feet from the trunks where the soil had not been tilled for many years had an average apparent specific gravity of 1.30 in the 0 to 12-inch depth and 1.34 in the 12 to 24-inch depth. The corresponding values for the samples taken at about the mid-point between the rows where tillage had been frequent were 1.47 and 1.33. In other words, the first foot of soil in the tilled area weighed about 10.5 pounds per cubic foot more than that in the untilled area, whereas the weights were about equal in the second foot of soil. At Delhi the apparent specific gravity of the first foot of Oakley fine sand was 1.54, the second foot, 1.40, and the third foot, 1.44. The Fresno sandy loam at Hughson had 1.47 for the first foot, 1.36 for the second, and 1.38 for the third foot. The first foot of Madera and Gridley loam had an apparent specific gravity of 1.30, the second and third foot each had 1.32. It seems that tillage on the sandy soils has greater effect in packing the soil than on clays. Obviously, volume-weight measurements made on the surface foot of some soils may not indicate the volume-weight of the deeper layers. The compacting of the soil in the first foot of the sandy soils may be a contributing cause for the high ratios for this depth of soil shown in table 2 and in figures 3 to 6. The slow rate of percolation on the Fresno sandy loam was particularly noticeable. The plots in this soil were irrigated on the afternoon of October 3. The first set of samples were taken on the morning of October 5, two days later, and the first

foot of soil was saturated. Drainage of free water out of the first foot continued until October 9.

A further illustration of the effect of soil structure on the downward movement of water is shown in figure 2. The irregular outline of the wetted area in the plot, to which a depth of 8 inches of water was added, is due to lens shaped masses of dense soil projecting upward from the hardpan layer. The low ratios of field capacity to moisture equivalent between and near these dry masses of soil probably are due to dry soil encountered in driving the soil-sampling tube into the soil, although these dry plots were not apparent on the face of the trench when the samples were taken. Somewhat the same condition was noticed in sampling the Yolo clay plots at Davis to which a depth of 8 inches was applied. The average ratio for the first foot, 9 days after irrigation, was only 0.88, and that of the second foot, 0.78, whereas the third and fourth feet were fully wetted as indicated by the ratios of 0.97. Some of the samples from the first 2 feet in this plot had ratios from 0.93 to 1.03 and our notes showed them to be marked "wet," but others were as low as 0.63 and were marked "dry." We believe that the failure to wet all of the upper 2 feet, while the lower layers were raised to the field capacity, even though the amount of water applied was large, was due to spots of compacted soil. The plot to which 12 inches of water was applied had the soil wetted throughout the entire depth sampled. Apparently the greater depth of water, in the latter case, resulted in holding the water on the plot long enough to allow the water to seep into the dense soil masses. The plots at Davis, which were irrigated on October 30, were located in a field which had grown a crop of barley during the previous winter and early summer. The field was not irrigated. In the past the field had been plowed and tilled when the soil was wet with resulting formation of "plow sole."

DISCUSSION AND CONCLUSIONS

The data presented here together with those of other investigators indicate that the moisture equivalent is a close measure of the field capacities of fine textured soils but not always of sandy soils. Our experiments show that the moisture equivalent can be used to indicate the field capacities of deep, drained soils with no decided changes in texture or structure, with moisture equivalents ranging from about 30 per cent down to about 12 or 14 per cent. We have not made field capacity tests with soils of finer texture than that indicated by a 35 per cent moisture equivalent. Below from 12 to 14 per cent, the moisture equivalent seems to be less than the field capacity. Inasmuch as the structure of the soil has also been shown to influence markedly the field capacity, this factor must likewise be considered in using such measurements.

The moisture equivalent seems to give a single-value determination closely related to texture, at least for the fine textured soils, in spite of the fact that the structure of the sample used in making the moisture equivalent determination may be changed from that found in the field.

Our results with the wilting of plants (10) have shown that the residual moisture content at permanent wilting, which we call the "permanent wilting percentage" does not bear a linear relation to the moisture equivalent. The ratios of moisture equivalent to permanent wilting percentage for more than 60 soils ranged from 1.4 to 3.8. Both high and low ratios were found with sands as well as with clays. It has been suggested that the moisture equivalent cannot be used as a measure of the relative moisture-holding property of soil. Our results clearly indicate, however, that it is a close measure of the field capacity of the fine textured soils, and hence, it may safely be assumed· that it is a relative measure for these soils. The ratios of moisture equivalent to permanent wilting percentage for our soils with moisture equivalents of less than 12 per cent ranged from 1.51 to 3.60 with an arithmetical mean of 2.55. The ratios of the field capacities of these sandy soils, if they were known, to the permanent wilting percentage probably would be higher than those just mentioned. Certainly, the use of the field capacity instead of the moisture equivalent as a measure of the relative water-holding power of the soil would increase the magnitude of the ratios. The average of these ratios would be much higher than the commonly used 1.84 ratio, if field capacities instead of moisture equivalents for these coarse textured soils were used.

The data given in table 2 indicate that the moisture content of the soil 2 or 3 days after irrigation, which was as close to the time of irrigation as samples could be taken, does not change materially in the next few days. Apparently there was downward movement from the second to the sixth day in the plots in Fresno sandy loam and in the Oakley fine sand plots. The compacted soil in the first foot of these plots, as is indicated by the high apparent specific gravities, probably caused water to accumulate above the plow sole and impede its downward movement. Of course, growing plants would begin to use water soon after it was applied to the soil and, as we have pointed out before, all of our tests in cropped fields show that the extraction of moisture by the plants is rapid enough to prevent appreciable downward movement after the first 2 or 3 days. We feel, therefore, that there is a rather definite soil-moisture content which if measured within 2 or 3 days after a rain or an irrigation may be assumed to be the field capacity, if there are no discontinuities in structure or texture present and in the absence of a water table. Furthermore, the moisture equivalent gives a fair measure of the fine textured soils, but not necessarily of the sands.

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THE EFFECT OF DRYING AND ULTRA-VIOLET LIGHT ON SOILS[1]

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It has been shown by numerous investigators that the drying or partial sterilization of soils may have marked effects upon their physical and chemical properties, as well as upon the crop producing power of these soils. The work herein reported was undertaken in 1927 to determine the relative effects of drying and treatment with ultra-violet light on the behavior of soils. Separate portions of a fine sandy loam soil were dried in the sunshine in the open, in sunshine under glass, in the shade, and in the oven at 100°C. Portions of soil dried in the sun and those dried in the shade as well as undried soil were treated with ultra-violet light. In treating with ultra-violet light the soil was spread out in a thin layer under a mercury vapor lamp and then at intervals of a few minutes a thin layer was removed from the upper surface with a straight edge thus exposing a fresh layer of soil. At frequent intervals the whole mass was mixed and the exposure continued. The ultra-violet light treatment of 2 kilos of soil varied from 15 minutes to 3 hours. The results reported in this paper were for the 2-hour treatments. These treated soils were compared in the laboratory and greenhouse.

BACTERIAL ACTIVITY

The ammonia content of the soil showed a slight initial increase, which was greater where the drying or treatment with ultra-violet light or a combination of the two had been most severe. After about 7 days the ammonia content decreased to a very low point.

The nitrate content of the soil was affected in the opposite direction to the accumulation of ammonia. The nitrate content became reduced immediately after treatment and then gradually increased for a period of several weeks. The average nitrate content as indicated by seven determinations over a period of 97 days following treatment showed a decided increase for the drying treatments and a still further increase where ultra-violet light was used in addition to the other treatments. A summary of these results is shown in table 1.

The nitrate-producing organisms seem to have been injured more by the treatments than were the ammonifying organisms, hence the rise in ammonia

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immediately after treatment, followed by a decrease as the nitrifying organism increased the nitrate content of the soil.

The total number of bacteria as determined on an agar media was reduced from 5,450,000 per gram in the untreated soil to about 3,170,000 in the soil treated with ultra-violet light, and to about 400,000 where dried in the sunshine. The ultra-violet light treatment, in addition to drying, reduced the numbers only slightly more than the drying treatments alone.

TABLE 1

Average nitrate content of soils during 97 days after treatment with ultra-violet light

NUMBER	TREATMENT	NO₂	INCREASE DUE TO ULTRA-VIOLET LIGHT
		p p m	*per cent*
1	Moist soil—untreated	44 1	.
2	Moist soil—ultra-violet light	63 7	44 4
3	Dried in shade	95 6	
4	Dried in shade and ultra-violet light	105 6	10 5
5	Dried in greenhouse	110 1	
6	Dried in greenhouse and ultra-violet light	119 2	8 3
7	Dried in sunshine	121 5	
8	Dried in sunshine and ultra-violet light	127 6	5 0

TABLE 2

Water-soluble calcium in soils with different treatments

NUMBER	TREATMENT	AVERAGE	INCREASE DUE TO ULTRA-VIOLET LIGHT
		p p m.	*per cent*
1	Moist soil—untreated	32 8	. . .
2	Moist soil—ultra-violet light	37 9	15 5
3	Dried in shade	45 1	. . .
4	Dried in shade—ultra-violet light	49 5	9 8
5	Dried in greenhouse	53 2	.
6	Dried in greenhouse—ultra-violet light	57 1	7 3
7	Dried in sunshine	59 2	. . .
8	Dried in sun—ultra-violet light	63 9	7 9
9	Dried in oven	86 2	. . . .

WATER-SOLUBLE CALCIUM

The amount of water-soluble constituents of a soil have been shown by a number of investigations to be considerably increased by drying. This was found to be the case in this work and a still further increase was obtained by exposure to ultra-violet light. This increase was slight but apparently consistent. Table 2 will give the average of seven determinations made over a period of 97 days.

It may be seen from these figures that the amount of water-soluble calcium was almost doubled where the soil was dried in the sunshine and treated with ultra-violet light. The effect of the light was greatest on the undried soil and then decreased as the severity of the drying increased. This may have been because, in spite of certain precautions, there was some drying of this moist soil while it was being exposed to the light treatment.

RATE OF SETTLING

Soils having the various treatments were mixed with five parts water and stirred in a mixing machine for 3 minutes, after which they were placed in tall glass cylinders and allowed to stand. The colloidal material in the dried samples settled out much more rapidly than did that in the untreated. The effect of ultra-violet light was greater on the dried samples than on the untreated, but in every case there was a marked increase in the rate of settling of the colloidal material due to the effects of the ultra-violet light. The explanation for this effect is not exactly clear but may be due in part at least to the dehydration of the colloidal materials and possibly to a reduction of the charge on the particles, as shown by Nordensen (3) with metallic colloids, due to the ultra-violet light. The increase in soluble salt content in the treated soils is another factor that would induce more rapid settling.

GROWTH OF PLANTS

Soils having the various treatments of drying and ultra-violet light were used in a limited number of tests for growing plants in the greenhouse. Very little effect was found for the various treatments on the growth of roots or tops of red clover or alfalfa. Previous work by Duley and Metzger[3] showed only very slight effect of ultra-violet light on wheat. The beneficial effect of drying has been reported by a number of workers, particularly by Lebedjantzev (2) in Russia, who found in many cases a distinct increase in the growth on soils that had been previously dried. Little effect of ultra-violet light was found on the number of nodules produced on alfalfa. This is similar to results since reported by Albrecht and Turk (1) who found that *B. radicicola* were not killed in a layer of soil $\frac{1}{16}$ inch thick and exposed to ultra-violet light for 4 hours.

Further tests of dried soils and those treated with ultra-violet light should be made to determine more definitely the effect on plant growth, particularly since the work of Lebedjantzev indicated that drying had a favorable influence. Such an effect might reasonably be expected since the nitrate content, soluble calcium content, and certain other chemical and physical properties of the soil were shown in this work to have been materially altered. It seems quite possible that the ultra-violet radiation contained in sunlight may be sufficient to have some slight effect upon the properties of field soils.

[3] Duley, F. L., and Metzger, W. H. Unpublished data. Kansas Agricultural Experiment Station.

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A SIMPLE ELECTRODIALYSIS CELL FOR THE ROUTINE DETERMINATION OF EXCHANGEABLE BASES IN SOILS

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The application of electrodialysis to soils by Sante Mattson (11) introduced a promising line of attack in base exchange investigations. In a preliminary study he showed that there was good agreement between the quantities of bases removed by electrodialysis and by extraction with N ammonium chloride and 0.05 N hydrochloric acid.

Unfortunately the types of electrodialysis cells so far developed are too complicated and costly to form part of the routine equipment for soil investigations. Mattson used a three-compartment cell, the center one being separated from the two outside ones by parchment paper, which formed the dialyzing membrane. The soil was placed in the central chamber and platinum gauze electrodes were placed in the solution in the two outside chambers, which were provided with outlets for the withdrawal of dialysates. The dialysis was carried out with a 220 volt D.C. current with a 25 to 50 watt lamp in series, and the solutions were periodically changed. The temperature rose to nearly 50°C.

Clark, Humfeld, and Alben (5, 7, 8, 9) developed in detail the technique used in the handling of this type of cell. Minor changes in construction were made, which were found to be a distinct improvement, a lower voltage of 110 volts was used with or without a lamp in series, and a cooling system of glass grids with a continuous flow of cold water inside each of the compartments was introduced. The temperature was thereby kept between 18 and 22°C. Later (8), considerable economy was effected by using copper as the cathode, and also simplifying the platinum anode. A sheet of carbon for the anode instead of platinum also gave good results for the bases, though on the acid side the solution became turbid, and was practically black from carbon separated from the electrode by the current.

The Mattson-type of cell has been used by Rost (13) with a 220 volt D.C. and a cooling device as was used by Humfeld and Alben. McGeorge used the original Mattson cell with a lower voltage, 50 to 75 volts.

The three-compartment cell described in the foregoing, although very valu-

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able for certain types of investigation, is not well suited for routine work.   If it is not necessary to separate the soluble anions from the soil, a two-compartment cell could be used for the separation of the bases,.the anode being placed in the same compartment as the soil.   This arrangement eliminates the resistance of one membrane, permits the electrodes to be placed more closely together, and greatly accelerates the dialysis.   Bradfield (2, 3), developed a type of cell suitable for routine work, based on the above considerations.   The soil sample is placed in a clean alundum extraction thimble (12.7 x 3.2 cm.) which is supported by a perforated nickel cylindrical cathode suspended in a specially constructed glass cell with a side arm.   The platinum foil anode is placed on the inside of the alundum thimble.   The electrodialyzate is drawn off from the side arm of the cathode chamber, the water being maintained at a constant level inside the cell by means of a siphon connected with a storage bottle in which a constant level is maintained.   After it has been started the apparatus is automatic.   The electrodialysis is continued until the dialyzate remains colorless when treated with phenolphthalein.   Usually a liter of dialyzate is obtained in about 4 to 8 hours.   The total bases are titrated by adding excess of standard acid, and back titration with standard alkali, after which the individual bases can be determined in the solution.

The Bradfield type of cell has also been used by Wilson (17, 18, 19, 20), in a series of investigations.

Crowther and Basu (6), using the Bradfield cell, found that still larger volumes than 1 liter are sometimes required for the complete removal of the bases. The dialyzate is made too dilute by the large amount of electroendosmose through the sides of the thimble in comparison with the transport of ions. This is eliminated to a large extent in the modified cell devised by Crowther and Basu, in which the space between the electrodes is reduced to a minimum, and the whole of the soil but none of the uncovered membrane lies directly between the two electrodes.   The membrane consists of a shorter and wider alundum R.84 thimble (5.5 cm. long and 3.5 cm. external diameter).   Ten grams of soil is placed on the flat bottom of the thimble and covered by a filter paper. The anode is a perforated disc of platinum or gold, and rests on the soil.   The thimble rests on a nickel cathode on the floor of a glass cathode chamber.   A constant water level is maintained in the anode chamber.   With 100 volts the temperature of the soil does not rise above 40°C. if the current is adjusted by an external resistance.   Electrodialysis is continued for about 1 hour after the dialyzate ceases to give a coloration with phenolphthalein.   Usually a volume of 500 to 700 cc. was sufficient for the complete removal of bases in 6 to 8 hours.   Traces of calcium and magnesium carbonates deposited on the outside of the thimble and in the cathode chamber are recovered by cleaning the inside of the thimble and then boiling it with water containing a known amount of 0.1 N acid and again with water.   The solutions are again used in turn to wash out the cathode chamber and are added to the dialyzate.   The total

bases are determined by back titration as described before, and the individual bases subsequently estimated.

The type of cell described in the foregoing, though the simplest so far developed, is nevertheless too expensive for a routine apparatus, and is considerably more complicated and costly than the cell described in the following.[2]

In the type of cell described in this paper, the cost of the outfit has been reduced to a minimum, chiefly through the simplicity of the electrodes, besides eliminating the outer cathode chamber. The whole outfit is within the means of any soil investigator.

THE NEW SIMPLE TWO-COMPARTMENT CELL

The principle of the cell described in this paper is based on the type of cell developed by Basu and Crowther (6), where the soil layer is reduced to a minimum in the form of a flat thin layer parallel to, and against, which is placed the anode. The cathode is placed below the flat bottom of the dialyzing vessel. The anode chamber consists of a special type of Jena glass filtering crucible, more or less like a Gooch crucible, in which the filter disc is made from sifted powdered glass, heated to its sintering point, and without any bonding material; the filter disc[3] is permanently sealed into the Jena glass.

The grade of porosity of the diaphragm found to be most suitable was No. 4., the average size of the pores of which was 5 to 10 u. The capacity of the vessel is 60 cc., diameter of diaphragm 4 cm., and height above diaphragm 5 cm. Since the filter plate is made of glass without any bonding material, it contains no exchangeable bases such as occur in most forms of alundum, which need preliminary treatment and are likely to absorb bases during use. The bottom of the crucible extends below the filter disc to nearly 1 cm., narrowing down slightly as shown in figure 1a. The filter disc forms the dialyzing membrane, and can be easily cleaned after use. The walls being made of glass are nonporous, therefore the endosmotic flow of water is through the filter disc only, so that the dialyzate obtained is not diluted.

The cathode consists of a circular piece of copper gauze fitted below the filter disc and close to it as shown on the diagram. This can easily be done by cutting the gauze slightly larger than the size of the filter plate, and working in the edges of the gauze closely toward the sides of the diaphragm by means of a small screw driver. Two layers of filter paper are first similarly placed between the filter plate and the copper gauze before the latter is fitted, for reasons explained later. The copper gauze is cut so that a portion of it extends below, bent as shown, so that it can be connected to the negative terminal. The connection is made through a hole pierced in the gauze through which the terminal is

[2] According to the catalogued price supplied by Messrs. Gallenkamp, six gold electrodes alone cost $45, whereas the corresponding nickel cathodes cost $17.

[3] These filters are supplied by Messrs. Gallenkamp & Co. Technico House, 17 to 29 Sun Street, Finsbury Square, London, E. C. 2., price 3/9. (See catalogue on "Fine Filtration and Ultra Filtration.")

attached in the form of a hook. After the cathode has been carefully fitted, the soil sample (10 gm. of a clay soil, or 25 gm. of a light soil) is placed in the crucible, and washed under suction with 200 cc. of 50 per cent alcohol, and
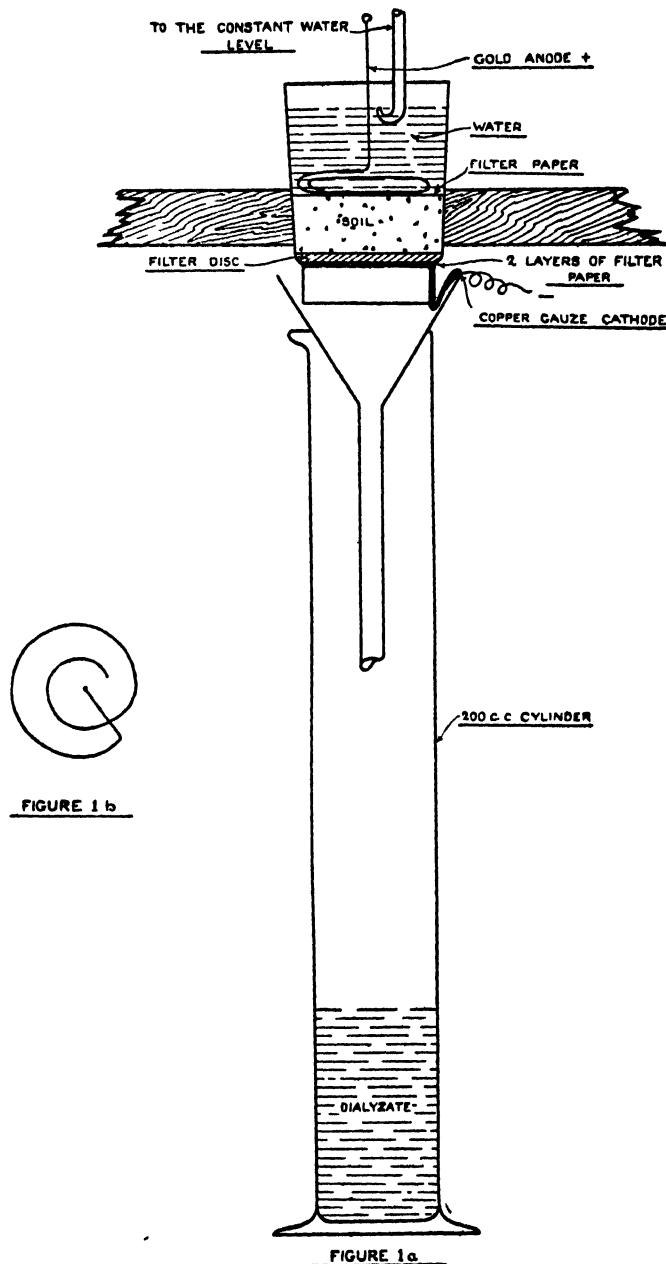

FIG 1. *a*, DIAGRAM OF THE NEW TWO-COMPARTMENT CELL, *b*, THE ANODE USED IN THE CELL

finally with 100 cc. distilled water, to remove soluble salts and especially chlorides. This is necessary as the traces of chlorides liberate chlorine during electrodialysis, which attacks the gold wire used as the anode. The anode consists of 10 inches of pure gold wire (English S.W.G. 24, .559 mm. diameter), bent in the form of a plane spiral of three turns as shown in figure 1b, placed parallel to, and lying on, the soil. It is preferable to place a layer of filter paper on the soil, and then the gold anode above, to prevent the layer of soil being disturbed by the water. Ten gold electrodes can be made from $12 worth of gold wire. The cell, after it has been fitted and is ready for use, rests in a hole in a wooden framework. The crucible is nearly filled with water and connected to a constant water level arrangement, after which the electrodes are connected to the terminals.

When the potential difference is applied there is an endosmotic flow toward the cathode of water which carries the cations to the filter disc, when they are liberated as hydroxides at the cathode. These are continuously removed by the endosmotic stream and drip into the funnel held below, and are placed in a 200-cc. measuring cylinder. After it has been started the process is automatic, the dialyzate being continually changed in the process.

In plate 1 are shown a set of 10 cells, mounted in two rows on a wooden framework, and connected in parallel to the main terminals. The anodes are connected to the insulated wire on top, and the cathodes are connected from below to the other insulated wire. The main terminals are connected to a D.C. of 200 volts and the external resistance is adjusted by means of a lamp of 15 to 100 watts in series depending on the number of cells in the circuit. The total initial current was rarely more than 200 milliamperes, but gradually fell toward the end; during the later stage, lamps of successively lower resistance were substituted.

The constant water level consists of a Marionette's bottle which is connected to the cells by means of T pieces and regulated in the usual manner.

The dialysis is continued for 1 hour after the dialyzate ceases to give a color with phenolphthalein.

EXPERIMENTAL

Soils examined

Four soil types were used in a critical study of the cell described in the foregoing:

Oxford clay: A heavy subsoil, decalcified, from top of brick-pit at Warboys, Huntingdonshire. Because of the proximity of the pit the land had not been cultivated for at least 40 years. pH 6.7.

Lower greensand: A light, market garden topsoil from Gamlingay, Cambridgeshire. pH 6.1.

London clay: Heavy clay topsoil from Purleigh, Essex. pH 6.6.

Fen peat: Peat topsoil from the Fenland of Kesteven, Lincolnshire. pH 4.3.

Methods

Ten grams of the Oxford clay, London clay, and peat, or 25 gm. of the lower greensand soils were used for electrodialysis.

All the following analyses were carried out in duplicate:

Total exchangeable bases.—The total exchangeable bases in the dialyzate were not determined by direct titration with standard acid as was done by Mattson, Bradfield, or Crowther and Basu. The technique used was slightly different, as traces of calcium and magnesium carbonates were deposited on the outside of the filter disc on the lower edges of the crucible and the funnel. Usually, especially from the peat soil, a brown deposit of iron hydroxide was formed on the filter plate. This was prevented by introducing two layers of filter paper between the gauze electrode and the filter plate as mentioned before, when this deposit, as also the traces of calcium and magnesium carbonate, were concentrated mainly on this filter paper. Instead of removing these traces with standard acid as was done by Crowther and Basu, they were removed by means of 10 per cent acetic acid. The copper gauze electrode was also left in acetic acid for a few minutes, and then washed with distilled water, the filter similarly treated, and the traces adhering to the filter plate and the edges of the crucible also were removed. The funnel and the collecting cylinders were washed in acetic acid and added to the bulk of the dialyzate, together with the other washings, which were all evaporated down on a water bath in a large porcelain dish. When the volume became small the residue was transferred to a 9-cm. porcelain dish in which the subsequent operations were carried out. When the evaporation was complete the dish was dried over a Rose burner and when dry finally ignited in a muffle furnace at a dull red heat for 5 minutes.

In this process the acetates are quantitatively converted to carbonates or oxides as shown by Rice Williams (16), and by Bray and Willhite (4). The bases were then estimated, using the details of Rice Williams. When cold, 50 cc. of 0.2 N hydrochloric acid was added, the solid being well rubbed in the acid with a rubber tipped glass rod. The dish was covered with a clock glass and allowed to stand over night. The contents of the dish were then filtered through a small filter paper into a 600-cc. beaker and washed with hot distilled water. When cold the excess acid in the filtrate was titrated with 0.2 N sodium hydroxide, using methyl red as indicator. Using standard sulfuric acid and boiling off the carbon dioxide after filtration gave the same results as in the aforementioned process, showing that when these details are used, no appreciable amount of carbon dioxide remains. The total bases were calculated in milligram equivalents from the volume of acid used.

Besides the fact that the filtrate so obtained after titration with standard alkali can be used without further treatment for the determination of the exchangeable calcium, magnesium, and potassium, the treatment is indispensable in the determination of the total bases of the dialyzate from the peat soil as it was colored brown by the humic matter.

Exchangeable calcium.—If magnesium is to be determined, the soluble silica

in the filtrate after titration is removed by evaporation to dryness and heating for 3 hours in a hot air oven below 120°C.[4]   The residue was taken up in hydrochloric acid and filtered.

To the filtrate 5 gm. ammonium chloride was added, the solution heated to boiling, 1 gm. oxalic acid added, and the calcium precipitated as oxalate by Blasdale's method (15), by adding 1 per cent ammonia, and being allowed to stand 4 hours.   The calcium in the precipitate was determined after washing by the volumetric permanganate method after solution in sulfuric acid.

Exchangeable magnesium.—In the filtrate from calcium oxalate the magnesium was precipitated by means of sodium phosphate and 10 per cent ammonia.   After standing 24 hours the precipitate was filtered, ignited, and estimated as the pyrophosphate.

Exchangeable potassium.—The potassium was determined in the dialyzate from another cell after titration of the total bases by the volumetric-cobaltinitrite-permanganate method, using the technique developed by Milne (12). Here the advantage of the preliminary acetic acid treatment and subsequent ignition becomes apparent.   This serves for the determination of the total bases, for the removal of the organic matter, and the sesquioxides for the potassium determination.   No further treatment was necessary besides evaporation to a small bulk.

Very satisfactory agreement between duplicates was obtained in all the foregoing determinations.

Exchangeable sodium was not determined.

Exchangeable bases by displacement.—The same amounts of soil as were used for electrodialysis were treated with N ammonium acetate, in preference to N ammonium chloride.   The advantages of ammonium acetate have been discussed by Schollenberger (14).   The total bases can be determined by converting the acetates to carbonates by ignition, followed by titration as before by the methods of Bray and Willhite (4), and Rice Williams (16).   The soils were leached up to a volume of 500 cc. with ammonium acetate adjusted to pH 7. The ammonium acetate was removed by volatilization on the water bath, and the total bases replaced as acetates were determined as before and subsequently the individual bases in the titrated solutions.

Hydrogen-ion concentration.—The pH values of the soils before and after electrodialysis were determined with a quinhydrone electrode and a soil-water ration of 1:2.

<div align="center">DISCUSSION</div>

<div align="center">*Exchangeable bases* ˙</div>

The quantities of exchangeable calcium, magnesium, and potassium extracted, when the soils were electrodialyzed or leached with N ammonium acetate, are shown in table 1.   The values are expressed in milligram equivalents per 100 gm. soil.

[4] J. W. Mellor: Treatise on Quantitative Inorganic Analysis, Vol. I.

TABLE 1

Exchangeable bases as determined by electrodialysis and by displacement with normal ammonium acetate

SOIL	MILLIGRAM EQUIVALENTS PER 100 GM SOIL															
	1 Calcium		2 Magnesium		3 Potassium		4 Ca, Mg, K		5 Total by titration		6 Difference 4–5		7 CaSO₄		8 Ca, Mg, K 4–7 corrected	
	Dialysis	Displacement	Dialysis	Displacement	Dialysis	Displacement	Dialysis	Displacement	Dialysis	Displacement	Dialysis	Displacement	Dialysis	Displacement	Dialysis	Displacement
Lower greensand	5 39	5 49	0 99	0 89	0 50	0 59	6 88	6 97	5 70	5 37	1 18	1 60		0 99		5 98
Oxford clay	24 22	24 36	3 46	3 08	0 76	0 84	28 44	28 28	25 56	25 12	2 88	3 10	1 70	3 26	26 74	25 02
London clay	21 21	21 16		6 66	0 92	0 82		28 64	24 27	26 74		1 90		2 66		25 98
Fen soil	38 72	36 57	1 76	1 76	0 53	0 69	41 01	39 02	35 77	35 70	3 24	3 32	2 47	5 18	38 54	33 84

A comparison of the individual bases as determined by the two methods employed shows these to be in close agreement. There is excellent agreement for calcium, whereas the agreement for magnesium and potassium is satisfactory, considering the small quantities of these bases estimated. In the case of magnesium, for the four soils examined, no discrepancies such as were recorded by Wilson (17) in the values obtained by the two methods employed are shown by our results. Wilson noted that considerably less magnesium was recovered by electrodialysis.

Similar agreement is shown in the values for the total bases by titration by the two methods, except for the slight discrepancy in the case of the Peat soil. The sum of the individual bases shown in column 4 shows similar agreement. When, however, the sum of the bases in column 4 is compared with the values for the total bases by titration given in column 5, very considerable discrepancies are noted, the values by titration being lower, as much as 12 per cent in the case of the Oxford clay. Such divergencies cannot be accounted for by experimental errors, therefore some other acid radical which does not give an oxide or carbonate on ignition must be present.

In these soils the likely radicals are sulfate, and perhaps phosphate. Such differences of the order recorded in column 6 have been noted by Rice Williams (16). In the four soils under discussion in this paper the differences were traced to sulfate. The ammonium acetate leachine liquid was evaporated and ignited, and the residue taken up in hydrochloric acid was examined for sulfate. The amounts recorded in column 7 were determined. Traces of phosphate were found in the extract of the Lower Greensand soil.

It may be noted that the amounts in column 7 are larger than the differences in column 4. This is because the values for exchangeable sodium have not been included in the sum of the bases determined individually. As it was found that sulfates are present in the soils, samples of Oxford clay and the peat soil in which the largest discrepancies were noted were again electrodialyzed, and after preliminary ignition and solution in hydrochloric acid, were examined for sulfates. The amounts found are recorded in column 7. The sulfuric acid is washed down into the basic dialyzate, possibly toward the end of the process, and reacting with the bases, lowers the titration value. Hence the value for exchangeable calcium is higher than the true figure, and the total bases by titration give the correct value for the total exchangeable bases, i.e., the S-value of Hissink.

The figures for calcium sulfate obtained by dialysis (column 7, table 1) are slightly lower than the corresponding values obtained by displacement, which is perhaps due to the fact that the dialyzates on which the sulfates were determined were obtained in 6 hours. The dialyzates on which the total titration values were determined were obtained in 14 hours with a slightly lower current and with a larger volume of water, which may have carried down more sulfuric acid.

Rate of dialysis and volume of dialyzate

Humfeld and Alben (8), using the modified Mattson cell, obtained complete dialysis in 45 hours.   Rost (13), found it necessary to continue the process for

TABLE 2

Rate of dialysis, volume of dialyzate, total bases extracts, and temperature of cells, using gold wire anodes and 60-cc. cells

SOIL	TIME*	VOLUME OF DIALYZATE	TOTAL BASES IN M E PER 100 GM SOIL	NUMBER OF CELLS IN CIRCUIT	TEMPERATURE	LAMP
		hours	cc.		°C	w.
London clay..............	(a) 11.5	200	27.15	2	29	25
London clay.	(a) 7 / (b) 3	250 / 200	26 9	3	22	25
	(a) 8 / (b) 2	250 / 60	27.50	3	23	25
	(a) 6.5 / (b) 3	200 / 150	27 4	3	23	
Greensand..............	(a) 11 / (b) 1.5	300 / 100	5 56	10	25	40
	(a) 14 5 / (b) 1 5	300 / 80	5.44	10	24	
	(a) 13 / (b) 1 5	380 / 150	5 88	10	25	
Oxford clay...	(a) 14 / (b) 1	380 / 20	25.40	10	24	40
	(a) 10 / (b) 2	180 / 50	25 4	10	29	40
	(a) 12 5 / (b) 2	450 / 50	25.60	10	29	40
Fen peat soil....	(a) 7.5 / (b) 2	330 / 360	38 50	6	25	40
	(a) 7.5 / (b) 2	260 / 560	36.70	6	25	40

* (a) = Time when dialysis was complete as indicated by phenolphthalein.
  (b) = Extra period for which dialysis was run.

the much longer period of 5 days.   These authors do not state for comparison the results obtained by displacement methods.   No data are given for the vol-

umes of the dialyzates obtained. Wilson (17), using the Bradfield two-compartment cell, continued the electrodialysis for 8 hours at an amperage of 100 milliamperes, and usually about 1 liter of the dialyzate was obtained. Except for magnesium, the values for the bases extracted by dialysis and by displacement showed close agreement. Crowther and Basu (6), using the Bradfield apparatus, found that still larger volumes than 1 liter are required for the complete removal of bases. But with their type of cell they found that a volume of 500 to 700 cc., removed in 6 to 8 hours, was sufficient for loams and clays, but 15 hours was necessary for light soils in which the initial current was very low, about 80 milliamperes.

TABLE 3

Rate of dialysis, volume of dialyzate, and temperature of cell

Results with carbon rod anode and 30-cc. cell

SOIL	TIME	VOLUME OF DIALYZATE	TOTAL BASES BY TITRA-TION*	NUMBER OF CELLS IN CIRCUIT	TEMPERATURE
	hours	*cc*			*°C.*
Fen soil (peat)	(a) 17.5	734	} 37.80	8	18–23
	(b) 4	226			
	(a) 17.5	470	} 37.90	8	20–26
	(b) 4	130			
London clay...............	(a) 17.5	222	} 26.5	8	22–31
	(b) 4	138			
	(a) 17.5	80	} 25.2	8	23–33
	(b) 4	30			
	(a) 17.5	145	} 25.3	8	23–28
	(b) 4	60			
	(a) 17.5	100	} 29 2	8	22–27
	(b) 4	38			

* M.E. per 100 gm. of soil.

The results obtained with the new cell described are given in table 2. No attempt was made to measure the current passing through each cell, but only the total current passing through all the cells. The time required was dependent on the current as shown by the fact that when few cells were run in the circuit less time was required for complete dialysis. When 3 cells were run at the same time with a 25 watt lamp in the circuit, the London clay was completely dialyzed in 7 to 8 hours. A soil of similar texture and of practically the same total base content, the Oxford clay, required from 10 to 14 hours when 10 cells were in the circuit with a 40 watt lamp. With 6 cells in the circuit with a 40 watt lamp the peat soil of high base exchange capacity was completely dialyzed

in 7 hours.   With clays and peat soils having a large amount of total bases, the initial current is higher than with light soils such as the Greensand, and the initial removal of the bases is very rapid.   However, the current soon falls with increasing resistance, and towards the end the rate of dialysis slows down. Though actual data of fractional dialysis are not given, most of the bases were removed in the first 3 hours in the case of clays and peats.   Ten cells were run at the same time, five with the Lower Greensand, and five with the Oxford clay soils, and though the initial current through the former was very small compared with the latter, dialysis was complete in both cases in nearly the same time—14 hours.   The initial current passing through all the cells was never more than 200 milliamperes, and dropped toward the end.

The volume of the dialyzate varied with the texture of the soil.   With heavy soils such as the Oxford and London clays, very concentrated dialyzates were obtained—dialysis was complete with 200 to 300 cc.   The current was run for an extra hour, however, and in this period the flow of water increased rapidly. This result is in contrast to that of Crowther and Basu (6), who used an alundum membrane and found that the rate of flow of the dialyzate steadily decreased with time.   Table 2 clearly shows this increase of flow toward the end, and the effect of permeability of the soil on the rate of flow.   With the Greensand soil about 300 cc. of dialyzate was obtained, whereas from the Peat soil comparatively large volumes were obtained, sometimes as much as 800 cc.

Table 3 records the results obtained with a 30-cc. cell, and a carbon rod as anode, and with a copper gauze cathode.   The carbon rod was of the ordinary uncored type used in arc lamps, and was suspended vertically in the soil.   Ten grams of soil was used for the dialysis, but although only 8 cells were run in the circuit, the current passing was very small because of the high resistance of the thick soil layer in these small cells.   The results obtained for the peat soils showed close agreement ·with those recorded in table 2, with the gold anode. Another disadvantage was the separation of fine carbon which passed into the pores of the filter plate, and would probably have rendered its life short.   The time taken for complete dialysis was 17 to 18 hours.

Temperature

A unique feature of the cell described is that there is no appreciable rise in temperature of the soil.   The current through each cell is small, as the total initial current with 10 cells was never more than 200 milliamperes.   The temperature never rose above 29°C., and was usually between 18 and 25°C.   Even when 3 cells were run, as long as the current was adjusted by a lamp, the temperature did not rise appreciably.

Hydrogen-ion concentration before and after electrodialysis

The pH values of the soils of the investigation before and after electrodialysis appear in table 4.   The Peat soil, though originally very acid (pH 4.27), contained the largest amount of bases because of the high absorption capacity of

the organic complex. The final pH values after complete removal of soluble anions ranged from 3.5 to 4.0, and are in conformity with the values obtained by Bradfield (2), Wilson (17), and others.

DECOMPOSITION OF THE ABSORPTION COMPLEX

As previously noted, in all cases a brownish deposit of iron and manganese hydroxides was formed on the bottom of the filter plate, and was later concentrated on the two layers of filter paper placed between the copper gauze and the filter disc. The amount was very small with all the soils except the peat. A colorimetric estimation showed the former to be the equivalent of about 0.0005 gm. mn and an equal amount of iron. With the peat soil, however, a considerable quantity of the brown deposit was concentrated on the filter papers, consisting mainly of iron hydroxide. McGeorge (10) states: "Large amounts of iron, aluminum and manganese did not make their appearance in the dialyzate until the calcium, magnesium, sodium and potassium were all completely removed." Humfeld and Alben (8) noticed a whiteish flocculent precipitate,

TABLE 4

Hydrogen-ion concentration of soils before and after electrodialysis

SOIL	pH BEFORE DIALYSIS	pH AFTER DIALYSIS	TOTAL BASES BY TITRATION*
Lower greensand	6 07	4 08	5.70
Oxford clay	6 70	3.71	25.56
London clay	6 55	3.51	27 24
Peat soil. . .	4 27	3 58	37.77

* M. E. per 100 gm. of soil.

apparently aluminum hydroxide, which appeared in increasing amounts as the solution tended to approach a neutral reaction, and with further dialysis and subsequent changes of solution the precipitate changed to a brown color, indicating increasing amounts of iron hydroxides, and possibly decreasing amounts of alumina. No such deposit was noted in the dialyzates obtained by the authors, except for the brown deposit on the filter papers.

This brings us to the much discussed question of the presence of iron, aluminum, and manganese in soils, as exchangeable components of the absorbing complex. McGeorge (10) tested this in detail by determining the exchange capacity of the soil before and after electrodialysis. No change in exchange capacity was observed, and he concludes that iron, aluminum, and manganese were not derived from the "zeolites," but rather were dissolved by the action of the hydrogen-ion upon the hydroxides and oxides present in the soil, and he questions the presence of these elements in an exchangeable form in soils. This question is at present under investigation by the authors, with special reference to the peat soil. The results of McGeorge indicate a highly stable exchange complex in soils.

SUMMARY

The various types of electrodialysis cells so far developed are unsatisfactory for routine work, and the cost of the apparatus is high. A simple and cheap two-compartment continuous-flow type of electrodialysis cell, well suited for routine determinations of the exchangeable bases of soils, has been described. A special type of filtering crucible is used as the anode chamber, with a filter plate made of sintered glass powder as the dialyzing membrane. The cost of the apparatus is greatly reduced by the use of a plane spiral of gold wire as the anode, and by the elimination of the outer chamber. Copper gauze is used as the cathode.

The cell is easily constructed, and has the following advantages: The soil layer is thin. There is no endosmotic flow of water through the sides, as these are of glass. The distance between the electrodes is short. The soil can be washed free of soluble salts before electrodialysis. Concentrated dialyzates are obtained. The cell can be easily cleaned. There is practically no rise in temperature during dialysis. Complete dialysis can be effected in 6 to 10 hours. A set of 10 cells complete with electrodes can be fitted up for about $20.

Results obtained with four types of soils are discussed. Very close agreement was obtained between the bases extracted by electrodialysis and those extracted by displacement with normal ammonium acetate.

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PLATE 1

TEN CELLS MOUNTED AND CONNECTED IN PARALLEL TO THE MAIN TERMINALS

THE INFLUENCE OF LIME ON THE RECOVERY OF TOTAL NITROGEN IN FIELD CROPS[1]

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The amount of nitrogen removed by field crops has long been a subject of much interest to students of agricultural chemistry. Nearly 100 years ago Boussingault (1) began a series of experiments on his farm at Bechelbronn in Alsace, where he introduced quantitative methods in field studies. He weighed and analyzed the manures used and the crops obtained, and at the end of the rotation drew up a balance sheet for the purpose of studying gains and losses of nitrogen.

About 50 years later Lawes and Gilbert (3) made a careful study of the nitrogen removed by various crops in a number of different rotations. They summarized their results as follows:

The average yield of nitrogen per acre per annum, was, with wheat, thirty-two years without manure, 20.7 pounds, and twenty-four years with a complex mineral manure, 22.1 pounds; with barley, twenty-four years without manure, 18 3 pounds, and twenty-four years with a complex mineral manure, 22.4 pounds; with root-crops, thirty-six years (including three of barley), with a complex mineral manure, 25 2 pounds; with beans, twenty-four years without manure, 31.3 pounds, and twenty-four years with a complex mineral manure, 45 5; with clover, six crops in twenty-two years, with one crop of wheat, three crops barley, and twelve years fallow, without manure, 30 5 pounds; with complex mineral manure, 39.8 pounds; with clover, on land which had not grown the crop for very many years, one year, 151.3 pounds; with a rotation of crops, seven courses, twenty-eight years, without manure, 36.8 pounds, and with superphosphate of lime, 45 2 pounds; with the mixed herbage of grass land, twenty years without manure, 33 pounds, and with complex mineral manure, including potash, 55.6; lastly, with Bokhara clover, five years, with mineral manure, between 80 and 90 pounds of nitrogen per acre per annum.

The root-crops yielded more nitrogen than the cereal crops, and the leguminous crops very much more still.

In all the cases of the experiments on ordinary arable land—whether with cereal crops, root-crops, leguminous crops, or a rotation of crops (excepting as yet the Bokhara clover)—the decline in the annual yield of nitrogen, none being supplied by manure, was very great.

Jenkins, Street, and Hubbell (2) found from nine series of tests with husking and silage corn at different experiment stations, that an acre of corn removed 87.5 pounds of nitrogen. In another experiment six tests gave for a 15-ton silage corn crop an average of 88 pounds of nitrogen an acre, and crops of husk-

ing corn at different places in Connecticut, calculated at 75 bushels an acre, gave an average of 92 pounds of nitrogen to the acre.

In the growing of continuous wheat with mineral fertilizers and legume green manure crops as a source of nitrogen, Lipman and Blair (5) found that wheat (including grain and straw) returned a 13-year average of 32.8 pounds of nitrogen an acre; with mineral fertilizers without nitrogen the return was 20.6 pounds of nitrogen an acre. Rye grown under the same conditions returned 34.9 pounds of nitrogen with mineral fertilizers and legume green manure, and 24.5 pounds with minerals only. In the annual report of the New Jersey station for 1923 (6), the same authors give figures to show that soybeans grown on well-limed soil returned through dry shelled beans and stalks, more than 100 pounds of nitrogen an acre. On unlimed land the average return for several varieties of beans was less than 15 pounds of nitrogen an acre.

In studying the problem of the removal of nitrogen by crops many factors must be considered. Among these may be mentioned climatic conditions, especially rainfall, the character of the soil, the soil reaction, the amount of fertilizer and lime used, cultural practices, etc.

In the work here reported an attempt is made to show the influence of two forms of lime on the nitrogen removed by the crops of some 5-year rotations.

<div align="center">EXPERIMENTAL</div>

For a period of more than 20 years nitrogen determinations have been made on grain, hay, forage, and vegetable crops, grown on plots where calcium and magnesium limestone have been used in comparative tests. From these determinations and the dry weights, the amount of nitrogen removed through the crops, has been calculated.

The grain crops have included corn, oats, wheat, and barley; the hay has usually been timothy and clover; the forage crops have included soybeans, cowpeas, winter vetch, oats and peas, rye, millet, sorghum, and rape; the vegetable crops have been tomatoes, cucumbers, lima beans, carrots, and beets.

In the majority of cases the crop samples were first dried and nitrogen determinations made on the dry material. In some cases vegetables were weighed fresh and nitrogen determinations made on the fresh sample. The total nitrogen removed through the crop has been calculated on the acre basis.

The two forms of lime have been used in four different rotation systems:

Rotation 1—Corn, small grain, hay
Rotation 2—Corn, potatoes, grain, hay
Rotation 3—Corn, vegetable crops
Rotation 4—Corn, forage crops

The limestone is used in amounts equivalent to 1,000, 2,000, and 4,000 pounds an acre. Including one plot without lime, this gives seven plots for each system or 28 one-twentieth acre plots. The limestone has been applied at intervals of 5 years, preceding the corn. The no-lime plots have become noticeably

TABLE 1

Total nitrogen recovered in a 5-year rotation—1908–1912

LIMESTONE TREATMENT	1908	1909	1910	1911	1912	5-YEAR AVERAGE
			Rotation 1			
	Corn	Oats	Wheat	Oats*	Timothy and clover	
	lbs	lbs	lbs	lbs	lbs	lbs
No limestone	38 5	17 6	27 5	30 1	16 3	26 0
½ ton Ca limestone	42 2	18 3	27 7	28 5	18 8	27 1
½ ton Mg limestone	56 1	24 8	38 5	40 0	25 7	37 0
1 ton Ca limestone	47 2	17 9	35 9	32 3	18 7	30 4
1 ton Mg limestone	51 5	27 4	38 7	38 9	28 4	37 0
2 tons Ca limestone	49 8	22 4	36 6	34 0	19 3	32 4
2 tons Mg limestone	52 4	29 6	38 9	43 0	29 8	38 7
			Rotation 2			
	Corn	Potatoes	Rye	Oats*	Timothy and clover	
	lbs	lbs	lbs	lbs	lbs	lbs
No limestone	44 0	3 7	32 4	35 7	24 8	28 1
½ ton Ca limestone	63 3	3 6	40 6	41 2	33 5	36 4
½ ton Mg limestone	50 3	3 6	44 4	39 7	31 5	33 9
1 ton Ca limestone	Lost	3 3	38 2	38 2	30 4	27 5†
1 ton Mg limestone	42 6	3 2	49 1	41 8	30 7	33 5
2 tons Ca limestone	55 5	3 4	47 6	36 0	36 1	35 7
2 tons Mg limestone	47 7	3 4	55 0	37 8	30 2	34 8
			Rotation 3			
	Corn	Potatoes	Tomatoes	Lima beans	Cucumbers	
	lbs	lbs	lbs	lbs	lbs	lbs
No limestone	41 5	2 4	24 2	19 5	5 8	18 7
½ ton Ca limestone	45 5	2 3	38 2	28 0	8 8	24 6
½ ton Mg limestone	42 7	2 9	34 2	26 3	8 3	22 9
1 ton Ca limestone	44 9	2 9	34 6	28 1	10 2	24 1
1 ton Mg limestone	49 8	3 4	34 3	27 7	5 8	24 2
2 tons Ca limestone	50 7	3 2	31 8	25 8	9 7	24 2
2 tons Mg limestone	53 5	2 8	39 0	24 6	5 0	25 0

TABLE 1—*Concluded*

LIMESTONE TREATMENT	1908	1909		1910		1911		1912		5-YEAR AVERAGE
	Corn	Oats and peas	Millet	Rape	Vetch	Rye	Cow peas	Oats and peas	Cow peas	
	lbs	lbs	lbs.	lbs	lbs.	lbs.	lbs	lbs	lbs.	lbs.
No limestone	37 6	38 2	15 9	23 0	43 5	41 2	49 2	38.6	25.9	62.6
¼ ton Ca limestone	39 8	40 3	16 8	30.8	59 9	43 8	62 3	58 2	32 0	76 8
¼ ton Mg limestone	47 9	51 7	16.5	32 5	93 1	42 6	35 3	48.7	27.1	79.1
1 ton Ca limestone	42 0	32 0	18 7	36 3	93 5	51 0	65 6	58 8	44 2	88 4
1 ton Mg limestone	46 2	51 7	19 6	35 4	101 2	37 4	45 4	53.0	34 3	84 8
2 tons Ca limestone	53 9	40 2	19 2	44 2	93 0	47 8	50 7	42 2	41 9	86.6
2 tons Mg limestone	37 7	40 5	20 5	33 3	114 4	39 6	47 9	48 6	42 0	84.9

* Timothy and clover failed; oats substituted.

† Four-year average.

acid, whereas those that receive 1,000 and 2,000 pounds an acre are moderately to slightly acid, and those that receive 4,000 pounds are about neutral or slightly alkaline. The lime requirement of this soil was originally about 1,200 to 1,400 pounds CaO an acre. The soil of these plots lies so close to the older Penn soils, that it can hardly be considered a typical soil, but it is classed as Sassafras loam.

FERTILIZER TREATMENT

The fertilizer treatment has varied slightly, but in the majority of cases superphosphate has been used at the rate of about 300 pounds an acre, and muriate of potash at the rate of 50 to 100 pounds an acre. For non-legume crops a nitrogenous fertilizer has been used to give the equivalent of about 150 to 200 pounds of nitrate of soda an acre. Only small amounts of nitrogen have been used for the legumes in the forage crops rotation. No farm manure has been used. Results for the four 5-year periods, for the four rotations are shown in tables 1 to 4.

Legume crops grown on the limed plots have almost invariably shown a higher percentage of nitrogen than those grown on the unlimed plots. In the majority of cases also they have shown a higher yield of dry matter. Thus with increase in percentage of nitrogen and also in yield, there has been a decided increase in the amount of total nitrogen returned per acre. In a number of cases also non-legume crops grown on limed plots have shown a somewhat higher percentage of nitrogen than those grown on the unlimed plot.

In the forage crops rotation two crops were grown during 4 of the 5 years,

TABLE 2

Total nitrogen recovered in a 5-year rotation—1913–1917

LIMESTONE TREATMENT	1913	1914	1915	1916		1917	5-YEAR AVERAGE

Rotation 1

	Corn	Oats	Wheat	Timothy and clover*		Timothy and clover*	
	lbs.	lbs	lbs.	lbs	lbs	lbs	lbs
No limestone	25 6	19 7	24 1	31 2	4 3	19 9	25 0
½ ton Ca limestone	33 1	17 0	28 3	41 3	25 0	28 5	34 6
½ ton Mg limestone	41 2	21 5	30 5	45 8	28 9	27 8	39 1
1 ton Ca limestone	40 3	22 2	31 5	52 7	35 9	30 9	42 7
1 ton Mg limestone	52 3	23 4	29 6	54 8	51 3	32 3	48 7
2 tons Ca limestone	49 3	20 8	28 7	52 6	45 0	29 7	45 2
2 tons Mg limestone	54 2	24 0	29 7	52 2	47 6	34 6	48 5

Rotation 2

	Corn	Potatoes	Rye	Timothy and clover*		Timothy and clover*	
	lbs	lbs	lbs	lbs	lbs	lbs	lbs
No limestone	33 3	22 5	28 2	39 0	14 9	27 3	33 0
½ ton Ca limestone	50 6	23 8	26 7	38 6	23 0	29 4	38 4
½ ton Mg limestone	56 5 ·	21 7	29 4	52 7	26 5	34 4	44 2
1 ton Ca limestone	61 2	20 9	29 5	56 3	26 5	39 0	46 7
1 ton Mg limestone	62 1	21 1	30 6	46 2	44 6	37 5	48 4
2 tons Ca limestone	70 4	20 3	28 8	62 2	39 6	36 0	51 5
2 tons Mg limestone	60 9	19 3	28 5	55 2	41 5	34 7	48 0

Rotation 3

	Corn	Potatoes	Tomatoes	Lima beans	Cucumbers	
	lbs	lbs	lbs	lbs	lbs.	lbs.
No limestone	43 0	15 4	40 6	16 7	9 5 ·	25 0
½ ton Ca limestone	49 1	16 2	48 1	22 4	14 8	30 1
½ ton Mg limestone	46 5	16 4	42 9	19 3	16 9	28 4
1 ton Ca limestone	53 4	19 1	46 9	29 4	18 3	33 4
1 ton Mg limestone	56 3	19 3	46 7	23 2	16 9	32 5
2 tons Ca limestone	55 4	20 7	43 1	22 8	15 0	31 4
2 tons Mg limestone	62 1	16 3	48 7	14 3	15 9	31 5

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TABLE 2—*Concluded*

LIMESTONE TREATMENT	1913	1914		1915		1916		1917		5-YEAR AVERAGE
	Corn	Oats and peas	Millet	Vetch	Rape	Rye	Cow peas	Oats and peas	Cow peas	
	lbs	lbs	lbs	lbs	lbs	lbs	lbs	lbs	lbs. *	lbs
No limestone	37 0	25 2	4 7	7 1	16 7	23 5	28 5	27 4	32 3	40 5
¼ ton Ca limestone	45 3	41 2	6 3	28 7	18 4	29 5	28 3	42 6	34 8	55 0
¼ ton Mg limestone	39 8	37 9	2 2	60 1	19 2	30 8	54 9	57 6	47 2	69 9
1 ton Ca limestone	52 1	41 6	8 8	49 6	22 1	28 4	41 1	49 0	46 7	67 9
1 ton Mg limestone	43 0	35 7	3 8	86 5	23 3	30 5	65 4	54 6	50 4	78 6
2 tons Ca limestone	55 4	46 8	7 3	65 8	24 4	36 1	59 7	56 1	64 9	83 3
2 tons Mg limestone	44 8	38 8	4 1	89 6	27 1	23 1	54 9	64 1	55 3	80 4

Rotation 4

* Two cuttings of timothy and clover.

with the exception of 1924. This makes 35 crops on these plots for the 20 years. In this connection it may be pointed out that the crops in this rotation have returned much more nitrogen than those of the other rotations. This is, of course, mainly because most of these crops were legumes and therefore got a part of their nitrogen from the air. It will be remembered that very little nitrogen has been applied for these crops. This is of special interest because it shows that where lime and minerals are used with care, yields may be kept up to a fair level over a period of years without the use of farm manure and with a minimum of nitrogenous fertilizers. With the exception of the check plot the average nitrogen returns for this rotation for the last 5 years are about as high as for the first 5 years.

FIRST 5-YEAR PERIOD

The figures for the first 5-year period are shown in table 1. Reference to the averages shows that excepting the check plot, rotations 1 and 2 returned about 30 to 35 pounds nitrogen to the acre. Rotation 3 returned about 24 pounds, and rotation 4 a little better than 80 pounds.

In nearly all cases the no-lime plots gave a lower nitrogen return than the limed plots, though in the case of potatoes and vegetables the difference is slight. In many cases the 1,000- and 2,000-pound applications have given about as high a nitrogen yield as the 4,000-pound application. The failure of the timothy and clover in rotations 1 and 2 in 1911 undoubtedly reduced the nitrogen yield for that year.

With the exception of rotation 1, the 5-year average indicates that the two forms of limestone had about the same effect with reference to the amount of

TABLE 3

Total nitrogen recovered in a 5-year rotation—1918-1922

LIMESTONE TREATMENT	1918	1919	1920	1921	1922	5-YEAR AVERAGE

Rotation 1

LIMESTONE TREATMENT	Corn	Oats	Barley*	Timothy hay†	Timothy hay†		5-YEAR AVERAGE
	lbs	lbs	lbs	lbs	lbs	lbs	lbs
No limestone	48 3	24 4	12 9	14 9	15 1	1 5	23 4
½ ton Ca limestone	54 6	34 4	25 3	22 3	30 2	2 5	33 9
½ ton Mg limestone	60 3	35 7	28 6	26 2	46 5	9 8	41 4
1 ton Ca limestone	55 8	35 8	26 1	23 9	52 7	7 7	40 4
1 ton Mg limestone	62 6	42 1	31 2	30 6	48 1	12 6	45 4
2 tons Ca limestone	57 7	36 0	28 9	24 5	61 8	14 7	44 7
2 tons Mg limestone	64 8	41 5	31 2	28 4	63 0	23 6	50 5

Rotation 2

LIMESTONE TREATMENT	Corn	Potatoes	Rye	Timothy hay†		Timothy hay†		5-YEAR AVERAGE
	lbs	lbs	lbs	lbs	lbs	lbs	lbs	lbs
No limestone	55 0	16 9	27 4	18 7	6 1	32 9	1 9	31 8
½ ton Ca limestone	52 3	23 8	29 3	20 3	11 0	51 2	13 3	40 2
½ ton Mg limestone	57 1	27 9	32 9	20 3	11 1	45 0	17 7	42 4
1 ton Ca limestone	63 7	26 8	31 5	21 2	23 3	67 4	20 3	50 8
1 ton Mg limestone	60 0	20 3	34 5	21 7	26 1	66 5	30 3	51 9
2 tons Ca limestone	67 3	23 3	37 2	18 0	17 3	58 4	21 7	48 6
2 tons Mg limestone	69 2	25 2	43 0	19 4	31 8	44 0	28 8	52 3

Rotation 3

LIMESTONE TREATMENT	Corn	Potatoes	Tomatoes	Lima beans	Cucumbers	5-YEAR AVERAGE
	lbs	lbs	lbs	lbs	lbs	lbs
No limestone	58 5	23 3	35 0	12 6	5 5	27 0
½ ton Ca limestone	68 7	29 0	28 9	19 8	9 4	31 2
½ ton Mg limestone	69 9	19 4	31 8	36 6	8 9	33 3
1 ton Ca limestone	72 0	26 8	26 3	31 9	10 4	33 5
1 ton Mg limestone	70 1	26 9	34 0	43 3	9 7	36 8
2 tons Ca limestone	72 0	22 7	19 9	49 2	13 1	35 4
2 tons Mg limestone	72 9	30 7	29 8	37 0	8 6	35 8

TABLE 3—*Concluded*

LIMESTONE TREATMENT	1918	1919		1920		1921		1922		5-YEAR AVERAGE
	Corn	Oats and peas	Millet	Rye and vetch	Rape	Rye and vetch	Soy-beans	Rye	Soy-beans	

Rotation 4

LIMESTONE TREATMENT	Corn	Oats and peas	Millet	Rye and vetch	Rape	Rye and vetch	Soy-beans	Rye	Soy-beans	5-year average
	lbs.	lbs.	lbs.	lbs	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
No limestone	59 4	27.2	9.3	17.8	15.1	33 7	27.3	19 0	31 9	48.1
½ ton Ca limestone	61 6	49 3	13 2	31 7	13 9	79 0	41 1	20 6	61 4	74 4
½ ton Mg limestone	62 8	44 3	10 8	40 8	7 0	92 1	64 5	28 2	59 4	82 0
1 ton Ca limestone	56 6	54 1	16 6	28 0	13 1	102 5	73 3	17 8	66 8	85 6
1 ton Mg limestone	58 3	43 3	11 4	62 5	11 7	115 1	75 9	32 3	82 9	98 7
2 tons Ca limestone	66 7	55 1	16 9	40 1	15 0	95 4	68 3	34 9	85 8	95 6
2 tons Mg limestone	56 5	44 6	12 6	54 3	15 3	101 9	70 6	21 2	96 1	94 6

* Wheat winter-killed; barley substituted.
† Clover seeded with timothy was largely a failure.

nitrogen returned in the crop. There are, it is true, many individual variations, but in the majority of cases the difference is not great.

SECOND 5-YEAR PERIOD

The total yields of nitrogen for the second 5-year period are shown in table 2. They do not differ greatly from those of the first 5 years. There were two cuttings of timothy and clover in 1916, and this gave a decided increase in nitrogen for rotations 1 and 2. The limed plots of these two rotations returned a 5-year average of about 45 pounds nitrogen to the acre. The 5-year average for the limed plots of rotation 3 is close to 30 pounds an acre, and the corresponding plots of the forage crop rotation gave about 72 pounds an acre.

THIRD 5-YEAR PERIOD

The results for the third 5-year period are shown in table 3. The general averages are quite close to the averages for the second 5-year period. The returns from the corn crop are, however, distinctly higher than for either the first or second 5-year periods. The 5-year average for the limed plots of rotation 1 is a little better than 40 pounds an acre; for rotation 2 it stands between 45 and 50; for rotation 3 it is close to 35, and for rotation 4 nearly 90 pounds an acre. This latter is equivalent in amount to over 500 pounds nitrate of soda an acre. In a majority of cases the 4,000-pound application of limestone gave the highest nitrogen returns, but it frequently happens that the increase with this amount over the yield with 2,000 pounds is only slight. The averages for this period indicate that the yields were slightly higher with the magnesian

TABLE 4

Total nitrogen recovered in a 5-year rotation—1923-1927

LIMESTONE TREATMENT	1923	1924	1925	1926		1927	5-YEAR AVERAGE

Rotation 1

LIMESTONE TREATMENT	Corn	Oats	Wheat	Timothy and clover*		Timothy	
	lbs	lbs	lbs	lbs	lbs	lbs	lbs
No limestone	37 5	23 7	34 1	12 5		20 8	25 7
½ ton Ca limestone	56 7	35 7	45 6	20 0	6 0	35 7	39 9
½ ton Mg limestone	66 7	36 7	50 0	22 9	26 0	50 3	50 5
1 ton Ca limestone	62 5	33 4	46 0	21 7	31 3	54 0	49 8
1 ton Mg limestone	70 1	36 9	45 0	28 3	36 9	52 4	53 9
2 tons Ca limestone	67 9	32 6	51 9	19 5	27 3	59 8	51 8
2 tons Mg limestone	71 2	36 5	38 8	28 9	41 7	62 7	56 0

Rotation 2

LIMESTONE TREATMENT	Corn	Corn	Oats	Timothy and clover*		Timothy	
	lbs	lbs	lbs	lbs	lbs	lbs	lbs
No limestone	47 4	50 2	26 9	19 7	2 1	25 7	34 4
½ ton Ca limestone	64 3	57 7	27 6	20 5	6 7	45 8	44 5
½ ton Mg limestone	64 3	59 4	29 2	21 3	18 1	59 5	50 4
1 ton Ca limestone	72 6	58 3	28 2	23 0	32 4	55 4	54 0
1 ton Mg limestone	67 7	66 2	29 5	24 4	27 5	60 9	55 2
2 tons Ca limestone	76 8	68 3	29 5	23 9	35 4	70 5	60 9
2 tons Mg limestone	73 5	70 0	30 1	27 5	30 8	48 7	56 1

Rotation 3

LIMESTONE TREATMENT	Corn	Corn	Rape	Sorghum	Beets	
	lbs	lbs	lbs	lbs	lbs	lbs
No limestone	63 3	53 8	42 9	29 6		37.9
½ ton Ca limestone	67 9	58 2	58 3	68 4	18 0	54 2
½ ton Mg limestone	66 9	50 3	54 1	105 8	22 3	59 9
1 ton Ca limestone	75 0	54 3	67 1	67 0	41 2	60 9
1 ton Mg limestone	73 7	52 0	67 4	71 7	31 3	59 2
2 tons Ca limestone	78 1	58 9	72 2	71 3	43 4	64 8
2 tons Mg limestone	70 5	57 8	83 5	70 5	34 3	63.3

TABLE 4—*Concluded*

LIMESTONE TREATMENT	1923	1924	1925		1926		1927		5-YEAR AVERAGE
	Corn	Oats and peas	Rye and vetch	Soy-beans	Rye and vetch	Soy-beans	Oats and peas	Soy-beans	
	lbs	*lbs*	*lbs*	*lbs*	*lbs*	*lbs*	*lbs*	*lbs*	*lbs*
No limestone	39 7	25 0	32 4	26 0	19 9	21 5	28 6	25 7	43 8
½ ton Ca limestone	56 1	42 3	36 4	51 8	22 5	47 4	36 1	48 0	68 1
½ ton Mg limestone	64 5	35 5	35 8	62 6	23 6	63 9	30 2	50 5	73 3
1 ton Ca limestone	60 7	39 2	36 6	63 9	20 4	81 1	42 9	55 7	80 1
1 ton Mg limestone	55 4	36 2	37 0	88 8	20 2	92 8	37 0	73 5	88 2
2 tons Ca limestone	67 6	35 5	45 7	84 5	27 5	86 2	43 9	89 4	96 1
2 tons Mg limestone	59 4	33 9	35 1	77 7	24 5	101 4	40 1	59 7	86 4

Centered: Rotation 4

* Timothy and clover, two cuttings

than with the calcium limestone. In the forage crop rotation the 4,000-pound application of limestone gave a nitrogen return of approximately 95 pounds an acre. Vegetables and potatoes usually give a rather low yield of nitrogen. The timothy and clover crop of 1921 was small and therefore gave a low yield of nitrogen.

FOURTH 5-YEAR PERIOD

The figures for the nitrogen returned through the crop during the fourth 5-year period are shown in table 4. It is interesting to note that the yields for rotations 1, 2, and 3 for this period are higher than for any of the three preceding periods. This is a fair indication that the nitrogen supply of the soil is being maintained, otherwise the yields would show a decrease rather than an increase. Here it may be pointed out that the vegetable rotation was changed for this period, only one vegetable crop—beets—being included.

The yields for the forage crop rotation have been well maintained, the general average for the limed plots being 82 pounds of nitrogen to the acre. In this rotation there is little difference between the nitrogen recovered with the two forms of limestone. Generally the returns with 4,000 pounds of the limestone are more than with either the 2,000 or 1,000 pounds.

YIELD OF DRY MATTER

The yields of dry matter (in some cases field weights of fresh vegetables) for the last 10 years are reported in tables 5 and 6, so that they may be studied in connection with the nitrogen data. The corresponding figures for the first 10 years have appeared in earlier publications (4, 7, 8).

TABLE 5
Yields of dry matter in a 5-year rotation—1918–1922

Rotation 1

LIMESTONE TREATMENT	CORN—1918 Grain	Stover	OATS—1919 Grain	Straw	BARLEY—1920 Grain	Straw	TIMOTHY HAY*—1921 First crop	Second crop	TIMOTHY HAY*—1922 First crop	Second crop	5-YEAR AVERAGE
	lbs.	lbs	lbs	lbs.	lbs.	lbs	lbs.	lbs	lbs.	lbs.	lbs.
None	2,328	1,820	720	1,600	452	588	1,700		1,600	136	2,189
½ ton Ca limestone	2,508	1,858	680	2,440	1,124	1,236	2,860		3,680	144	3,306
½ ton Mg limestone	2,716	2,056	720	2,400	1,144	1,476	3,420		4,040	660	3,726
1 ton Ca limestone	2,752	1,958	560	2,520	1,080	1,340	2,760		4,520	464	3,591
1 ton Mg limestone	2,620	2,314	680	2,720	1,216	1,584	3,680		4,800	810	4,085
2 tons Ca limestone	2,632	2,052	720	2,560	1,196	1,384	2,840		4,480	870	3,747
2 tons Mg limestone	2,988	2,208	640	2,720	1,200	1,420	3,560		4,560	1,480	4,155

Rotation 2

LIMESTONE TREATMENT	Grain	Stover	POTATOES† Primes (bu)	Seconds (bu)							4-YEAR AVERAGE
None	2,580	1,868	80 0	10 2	1,096	2,824	2,830	34	3,480	120	3,708
½ ton Ca limestone	2,468	2,140	81 1	12 5	1,280	3,520	3,220	128	4,560	800	4,529
½ ton Mg limestone	2,620	2,242	89 8	16.5	1,356	3,404	2,640	240	4,080	1,100	4,421
1 ton Ca limestone	2,972	2,310	97 8	13 8	1,212	3,868	2,970	600	5,440	1,360	5,183
1 ton Mg limestone	2,660	2,140	97 4	13 2	1,392	3,608	2,630	840	4,160	1,920	4,838
2 tons Ca limestone	3,032	2,238	82 8	13 2	1,440	4,240	2,320	520	4,360	1,320	4,868
2 tons Mg limestone	2,944	2,212	84 5	15 8	1,644	4,396	2,400	1,100	3,920	1,800	5,104

Rotation 3

LIMESTONE TREATMENT	Grain	Stover	Grain	Straw	TOMATOES† (lbs)	LIMA BEANS, DRY (lbs)	CUCUMBERS† (lbs)	
None	2,816	1,968	101 1	18 3	18,728	380	5,218	.....
½ ton Ca limestone	2,904	2,374	147 8	24 1	15,034	620	8,948	.....
½ ton Mg limestone	2,736	2,200	69 7	20 8	16,664	1,050	9,228	.....
1 ton Ca limestone	3,064	2,468	137 8	22 1	13,768	950	9,496	.....
1 ton Mg limestone	2,944	2,362	165 8	25 0	17,747	1,330	9,110	.....
2 tons Ca limestone	3,108	2,286	106 0	18 5	9,899	1,370	12,726	.....
2 tons Mg limestone	3,100	2,286	146 4	26 2	16,352	950	8,408	....

TABLE 5—*Concluded*

LIMESTONE TREATMENT	CORN—1918		OATS AND PEA HAY	MILLET	RYE AND VETCH	RAPE	RYE AND VETCH	SOY-BEAN HAY	RYE	SOY-BEAN HAY	4-YEAR AVERAGE
	Grain	Stover									
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.

Rotation 4

None	2,600	1,968	1,960	800	2,360	1,840	2,950	2,120	1,800	2,120	4,104
½ ton Ca limestone	2,752	2,222	3,640	1,040	2,840	1,168	4,660	2,900	2,800	3,120	5,428
½ ton Mg limestone	2,668	2,166	3,240	800	3,080	520	5,180	3,700	3,200	3,080	5,527
1 ton Ca limestone	2,760	2,134	3,640	1,160	2,200	1,172	4,988	3,220	3,120	2,960	5,471
1 ton Mg limestone	2,620	1,868	3,200	800	3,800	1,020	5,080	4,520	3,240	3,240	5,878
2 tons Ca limestone	2,964	2,166	3,920	1,000	3,440	1,120	5,292	3,880	4,120	3,160	6,212
2 tons Mg limestone	2,488	2,066	3,000	920	3,440	1,140	5,000	3,620	3,160	3,240	5,615

* Clover seeded with timothy was largely a failure.
† Field weights—not included in averages.

Table 5, which gives results for the 5 years 1918–1922, shows average annual yields for the limestone treated plots, varying from about 3,500 to 6,000 pounds total dry matter an acre. The largest total yields are found in rotation 4 where in 4 out of the 5 years two crops were grown each year.

Generally, the averages show an increase for the limed plots over the plots without lime amounting to more than 1,000 pounds an acre. In some cases this increase amounts to 2,000 pounds or more.

The results with the two forms of limestone do not always agree closely, but the differences are not consistently in favor of either one.

The yields for the 5 years 1923–1927 are shown in table 6. With the exception of rotation 1, the averages are slightly lower than for the preceding 5 years. Here also as in the preceding 5 years the limed plots show a decided increase over the plots without lime. Also the yields with the two forms of lime are generally in fair agreement.

THE PERCENTAGE OF NITROGEN IN DRY MATTER

Table 7 shows the percentage of nitrogen in the crops of the four rotations for the period 1923–1927 inclusive. These figures are of interest as showing the amount of nitrogen removed by 100 pounds of dry matter in the various crops.

It will be noted that the grains and soybean hay are comparatively high in nitrogen; corn stover contains approximately 0.6 to 0.9 per cent nitrogen; oats straw contains almost as much as the stover, whereas wheat straw is low in nitrogen. The low percentage of nitrogen in the timothy and clover (first cutting) indicates that there was very little clover with the timothy. A nitro-

TABLE 6

Yields of dry matter in a 5-year rotation—1923-1927

LIMESTONE TREATMENT	CORN—1923		OATS—1924		WHEAT—1925		TIMOTHY AND CLOVER—1926		TIMOTHY HAY—1927	5-YEAR AVERAGE
	Grain	Stover*	Grain	Straw	Grain	Straw	First crop	Second crop		
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs	lbs	lbs.	lbs.
Rotation 1										
None	1,740	2,188	671	1,346	1,053	2,644	1,242	. .	1,887	2,554
½ ton Ca limestone	2,483	2,406	1,108	1,950	1,545	4,306	2,476	230	3,789	4,059
½ ton Mg limestone	2,860	3,270	1,223	2,170	1,671	4,601	2,578	1,058	5,059	4,898
1 ton Ca limestone	2,973	3,010	1,041	2,038	1,642	4,421	2,370	1,210	5,320	4,805
1 ton Mg limestone	2,959	2,855	1,190	2,194	1,566	4,421	2,871	1,393	5,165	4,923
2 tons Ca limestone	2,920	3,031	1,037	2,108	1,668	4,777	2,039	1,072	5,433	4,817
2 tons Mg limestone	2,903	2,829	1,134	2,101	1,400	3,863	2,801	1,654	5,447	4,826
Rotation 2			CORN		OAT HAY					
None	2,315	2,765	2,463	2,110	1,620		2,550	80	3,253	3,431
½ ton Ca limestone	2,793	2,884	2,700	2,825	1,860		2,561	264	4,424	4,062
½ ton Mg limestone	2,828	2,904	2,813	3,180	1,800		2,846	603	5,094	4,414
1 ton Ca limestone	2,903	3,108	2,650	2,885	1,760		2,469	1,173	5,137	4,417
1 ton Mg limestone	2,643	3,017	3,013	3,000	1,860		2,585	965	5,701	4,557
2 tons Ca limestone	2,863	3,855	2,788	2,770	1,880		2,656	1,326	5,278	4,683
2 tons Mg limestone	2,835	3,341	3,050	2,995	1,880		2,705	1,048	4,396	4,450
Rotation 3					RAPE		SORGHUM		CARROTS**	4-YEAR AVERAGE
None	2,213	3,284	2,538	2,535	1,700		2,209		300	3,620
½ ton Ca limestone	2,660	2,606	2,638	2,805	2,400		5,109		2,440	4,555
½ ton Mg limestone	2,425	3,407	2,363	2,800	2,350		7,211		5,320	5,139
1 ton Ca limestone	2,803	3,775	2,550	2,915	2,800		4,883		8,960	4,932
1 ton Mg limestone	2,870	3,330	2,725	2,495	2,850		4,996		8,600	4,817
2 tons Ca limestone	2,675	3,971	2,750	2,735	2,900		5,073		10,720	5,026
2 tons Mg limestone	2,730	2,913	2,575	2,715	3,050		5,483		10,440	4,867

TABLE 6—*Concluded*

Limestone treatment	CORN—1923		OATS AND PEA HAY	RYE AND VETCH†	SOY-BEAN HAY	RYE AND VETCH	SOY-BEAN HAY	OATS AND PEA HAY	SOY-BEAN HAY	5-YEAR AVERAGE
	Grain	Stover*								
	lbs	lbs	lbs	lbs	lbs.	lbs.	lbs.	lbs.	lbs	lbs
Rotation 4										
None	1,628	1,764	1,757	3,800	1,220	2,374	1,682	2,466	1,294	3,597
½ ton Ca limestone	2,320	2,396	2,584	4,040	2,220	2,723	2,303	2,985	2,116	4,737
½ ton Mg limestone	2,745	2,998	2,570	4,040	2,560	2,723	2,710	3,083	2,166	5,119
1 ton Ca limestone	2,460	2,628	2,476	3,880	2,720	2,370	2,992	3,542	1,996	5,013
1 ton Mg limestone	2,713	3,095	2,552	4,240	3,160	2,159	2,971	3,267	2,367	5,305
2 tons Ca limestone	3,213	2,818	2,387	5,000	3,200	3,086	2,985	3,775	2,533	5,799
2 tons Mg limestone	2,730	3,217	2,376	3,920	2,960	2,356	3,189	3,436	2,035	5.244

* Cobs not included.
† Mostly rye.
** Field weights—omitted from average

gen percentage above two and one-half for the second cutting indicates clover as the predominant crop. The rye and vetch show a low percentage of nitrogen because there was very little vetch with the rye.

SUMMARY

Over a period of 20 years nitrogen determinations have been made on a variety of field crops grown on four separate 5-year rotations, with varying amounts of calcium and magnesian limestone.

The total nitrogen thus recovered has been calculated and reported for four 5-year periods.

The highest returns have been from the forage crops rotation where two crops a year—mostly legumes—have been grown 3 or 4 years out of the 5. Generally the corn crops have given the next highest returns and the vegetable and potato crops the lowest.

The yields of timothy and clover were low in 1912, 1917, and 1921, and consequently this influenced the yield of total nitrogen.

In most cases the yield of nitrogen was almost as high with 2,000 pounds of the limestone as with 4,000 pounds.

Taking the 5-year averages for the different rotations, the yields of nitrogen are very nearly the same with the calcium and magnesian limestone, though in some cases the latter gave slightly higher yields.

For the most part the yields of nitrogen are higher for the fourth 5-year period than for the earlier periods. This would indicate that the general

TABLE 7

Nitrogen in the crops of a 5-year rotation—1923-1927

LIMESTONE TREATMENT	CORN—1923		OATS—1924		WHEAT—1925		TIMOTHY AND CLOVER—1926		TIMOTHY HAY —1927
	Grain	Stover	Grain	Straw	Grain	Straw	First crop	Second crop	
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent

Rotation 1

LIMESTONE TREATMENT	Grain	Stover	Grain	Straw	Grain	Straw	First crop	Second crop	Timothy Hay
None	1 29	0 641	1 98	0 774	2 33	0 360	1 00		1 10
½ ton Ca limestone	1 47	0 789	2 14	0 616	2 17	0 281	0 81	2 60	0 94
½ ton Mg limestone	1 46	0 720	2 12	0 493	2 14	0 309	0 89	2 46	1 01
1 ton Ca limestone	1 44	0 609	2 18	0 528	2 08	0 267	0 91	2 59	1 02
1 ton Mg limestone	1 52	0 830	2 13	0 528	2 08	0 281	0 99	2 65	1 02
2 tons Ca limestone	1 52	0 726	2 11	0 510	2 27	0 295	0 96	2 55	1 10
2 tons Mg limestone	1 55	0 868	2 11	0 598	2 01	0 276	1 03	2 52	1 15

Rotation 2

LIMESTONE TREATMENT	Grain	Stover	CORN Grain	CORN Stover	OAT HAY	First crop	Second crop	
None	1 39	0 521	1 35	0 756	1 66	0 77	2 57	0 79
½ ton Ca limestone	1 56	0 656	1 40	0 654	1 48	0 80	2 55	1 04
½ ton Mg limestone	1 47	0 742	1 34	0 647	1 62	0 75	3 00	1 17
1 ton Ca limestone	1 60	0 783	1 30	0 782	1 60	0 93	2 76	1 08
1 ton Mg limestone	1 55	0 846	1 37	0 774	1 59	0 95	2 85	1 07
2 tons Ca limestone	1 58	0 789	1 53	0 891	1 57	0 90	2 67	1 34
2 tons Mg limestone	1 54	0 852	1 55	0 709	1 60	1 02	2 94	1 11

Rotation 3

LIMESTONE TREATMENT	Grain	Stover	Grain	Straw	RAPE	SORGHUM	BEETS*	BEET TOPS*
None	1 55	0 852	1 33	0 738	2 53	1 34		
½ ton Ca limestone	1 57	0 956	1 38	0 727	2 43	1 34	0 208	0 302
½ ton Mg limestone	1 58	0 805	1 31	0 654	2 30	1 47	0 233	0 376
1 ton Ca limestone	1 57	0 783	1 41	0 582	2 40	1 37	0 202	0 327
1 ton Mg limestone	1 50	0 877	1 34	0 582	2 36	1 44	0 210	0 330
2 tons Ca limestone	1 58	0 868	1 33	0 764	2 49	1 41	0 196	0 354
2 tons Mg limestone	1 57	0 909	1 36	0 793	2 74	1 29	0 220	0 301

TABLE 7—Concluded

Limestone treatment	CORN—1923		OATS AND PEAS	RYE AND VETCH†	SOY-BEANS	RYE AND VETCH	SOY-BEANS	OATS AND PEAS	SOY-BEANS
	Grain	Stover							
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Rotation 4									
None	1 52	0 805	1 43	0 852	2 13	0 84	1 28	1 16	1 98
½ ton Ca limestone	1 45	0 884	1 64	0 900	2 33	0 83	2 06	1 21	2 27
½ ton Mg limestone	1 47	0 773	1 38	0 886	2 45	0 87	2 36	0 98	2 33
1 ton Ca limestone	1 49	0 868	1 58	0 942	2 35	0 86	2 71	1 21	2 79
1 ton Mg limestone	1 36	0 562	1 42	0 872	2 81	0 94	3 12	1 13	3 10
2 tons Ca limestone	1 41	0 742	1 49	0 914	2 64	0 89	2 89	1 16	3 53
2 tons Mg limestone	1 37	0 641	1 43	0 895	2 62	1 04	3 18	1 17	2 93

* Fresh materials not dried
† Very little vetch in this crop.

fertility level of the soil is being maintained, and perhaps slightly raised, under the systems of cropping employed.

In nearly all cases unlimed plots returned less nitrogen and a lower crop yield than the limed plots.

In the case of legume crops the limed plots almost invariably yielded dry matter with a higher percentage of nitrogen than the unlimed plots.

Tables showing the yields of dry matter for the four rotations for the 10-year period 1918–1927, are included; also a table giving percentage of nitrogen in the dry matter of the different crops for the 5-year period 1923–1927.

Attention is called to the fact that no farm manure has been used and that the amount of commercial fertilizer applied has been moderate. Note is made also of the fact that the yields, as indicated by the total nitrogen returned, have increased slightly.

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DOUBLE INFECTION OF LEGUMINOUS PLANTS WITH GOOD AND POOR STRAINS OF RHIZOBIA[1]

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The demonstration that strains of Rhizobia vary in their ability to aid the growth of the leguminous plant raises many questions of practical importance. Certain strains of the pea nodule bacteria (5) and of the clover nodule organism (1) have been shown to be essentially parasitic. These ineffective or poor strains[2] seem to be widely distributed in nature; their presence in a field may be responsible for low yields or even crop failure, according to Leonard (9). The early studies relating to this problem are summarized by Hiltner (6). Knowledge of the relative infective power of the good and poor strains of the nodule organism and of the reaction of the host plant upon infection with these types is essential to any attempt to evaluate the losses due to such parasitic strains and to any program looking toward the replacement of the poor strains in the field with better ones. The investigations reported here give an answer to some of these questions.

ISOLATION STUDIES ON PLANTS INOCULATED WITH BOTH GOOD AND POOR STRAINS

A number of investigators have reported the simultaneous presence of two or more strains of the nodule bacteria on a single host plant. All of these reports, however, have been based either upon cultural, morphological, or serological differences in the strains rather than on their efficiency in aiding plant growth.

Greig-Smith (4), in 1906, reported isolating culturally distinct strains of Rhizobia from a single nodule of lupine; and in 1907 de Rossi reported the presence of two types of the nodule organism in plates made from a single nodule (11). In neither case is adequate proof given that the organisms found were actually the nodule organism. Beijerinck (2) stated that with lupine and

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[1] Published with the approval of the director.

[2] Definitions of certain terms as used in this paper are as follows:

Inoculation—the act of applying the Rhizobium to seed, soil, or plants

Infection—the entrance of the Rhizobium into the plant with the production of nodules on the plants.

Effective or good strain—a strain of Rhizobium capable of infecting and of aiding the growth of a particular host plant when it is grown in a nitrogen-deficient medium.

Ineffective or poor strain—a strain of Rhizobium capable of infecting but giving little or no aid to the growth of a particular host plant when it is grown in a nitrogen-deficient medium.

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serradella there are different kinds of bacteria isolated from the nodules at different seasons of the year.   His method of distinguishing between the types is not explained.

Serological investigations have offered a definite method of distinguishing between strains of the same species and have been used by several workers. Bialosuknia and Klott (3) found two strains belonging to different serological groups on a single plant of broad bean.   Okawara and Yoshida (10) reported the same phenomenon with peas and vetch.   A similar report, was made by Jimbo (8) working with Robinia.   Israilsky (7), on the contrary, failed to find more than one type of nodule organism on a single plant, and stated that, after being infected by one strain of the organism, the plant is not susceptible to infection by another type.   This agrees with the earlier views of Hiltner (6), who believed that only strains of higher virulence could invade plants already bearing nodules.

W. B. Sarles, of this station, in some preliminary experiments on this problem found it possible to cause simultaneous infection of soybeans with two serologically different strains of *Rhizobium japonicum* and in one instance found two strains in a single nodule.

A personal communication from Gray at the Rothamsted Experimental Station states that two different strains of the nodule organism were isolated from a single nodule of alfalfa.   Coalescence of two nodules which started their growth from closely adjacent root hairs may occur.   The presence of two different strains of nodule bacteria in such a nodule might be expected.

Technic

To test the possibility of a single plant's bearing nodules formed by both effective and ineffective strains, plants of alfalfa, clover, peas, and soybeans were grown under carefully controlled conditions and inoculated with representative effective and ineffective strains.   Later, isolations were made from a number of the nodules to recover the strains.   With each host plant the following series were carried:

   I. Uninoculated
   II. Inoculated at planting with the poor strain
   III. Inoculated at planting with the good strain
   IV. Inoculated at planting with both strains
   V. Inoculated with the poor strain at planting and 4 to 6 weeks later inoculated with the good strain.
   VI. Inoculated with the good strain at planting and 4 to 6 weeks later inoculated with the poor strain.

In series V and VI, at the time of the second inoculation, the pots were allowed to dry almost to the wilting point of the plants, and a heavy inoculation was made on the surface of the sand and immediately washed in with sterile water.

The seeds were treated with mercuric chloride to render them bacteria-free and were then planted in a sterilized glacial sand, low in nitrogen and of an

alkaline reaction. The plants were watered with sterile distilled water. Mineral nutrients were supplied at intervals using a modified Crone's solution.[3] The soybeans and peas were grown in open half-gallon pots, and the clover and alfalfa in cotton-stoppered glass bottles with duplicate sets in open half-gallon pots. All plants were grown in a greenhouse reserved for this work, and every precaution was taken to avoid contamination with other strains of Rhizobia.

TABLE 1

History and description of the cultures used

CULTURE NUMBER	HOST ADAPTATION	DATE OBTAINED	GROWTH ON MANNITOL YEAST-WATER AGAR	FERMENTATION CHARACTERS	LIMITING pH IN A PEPTONE, SUCROSE SOLUTION
Rhizobium meliloti					
100	Good	1912	Raised growth, considerable slime	Acid forming*	
101	Poor	1915	Thin growth, little slime	Non-acid forming	
Rhizobium trifolii					
202	Poor	1921	Medium slime, unevenly opaque	Acid forming	
205	Good	1922	Considerable slime, evenly opaque	Non-acid forming	
Rhizobium leguminosarum					
310	Poor	1924	Unevenly opaque	Non-acid forming	
313	Good	1924	Evenly opaque	Acid forming	
Rhizobium japonicum					
501	Good	1918	Moderate growth		4 4
502	Poor	1921	Thin growth		5 0

* The terms *acid forming* and *non-acid forming* are relative only. The *Rhiz. meliloti* were tested against lactose, glycerol, and mannitol, *Rhiz. trifolii* against glycerol and mannitol and *Rhiz. leguminosarum* against mannitol.

Two strains each of *Rhizobium meliloti*, *Rhiz. trifolii*, *Rhiz. leguminosarum* *Rhiz. japonicum*, which were tested on alfalfa, red clover, garden pea, and soybean respectively, were selected to give as great a contrast as possible in their effect on the host plant and in their cultural characteristics. A description of these cultures is given in table 1. In addition to the cultural differences listed in the table, the two strains of each species were serologically distinct, and the

[3] The following salts were ground together and used in the proportion of 1½ gm. per liter of distilled water. After standing 24 hours, the clear solution was decanted and sterilized before use. KCl–100 gm., $CaSO_4 \cdot 2H_2O$—25 gm., $MgSO_4 \cdot 7H_2O$—25 gm., $Ca_3(PO_4)_2$—25 gm., K_2PO_4—25 gm.

agglutination reaction was used to differentiate the strains. The various differences between the two strains in each species were great enough to make differentiation positive and relatively easy.

Isolations

A summary of the isolations made from these plants is given in table 2. A typical plant was selected from each pot or jar, and six or more nodules were plated out from it using a yeast-water mannitol agar. Where inoculations had been made with the two strains, an attempt was made to select nodules characteristic of each type. The earlier experiments on strain variation (1, 5) have all called attention to the fact that with the ineffective strains the nodules are small and widely scattered over the root system, whereas with the effective

TABLE 2

Strains of Rhizobia recovered from nodules after double inoculation of alfalfa (Medicago sativa),
red clover (Trifolium pratense), pea (Pisum sativum), and soybean (Soja max)

SERIES	INOCULATION PLAN*	ALFALFA	CLOVER	PEA	SOYBEAN
I	Control	. . ..	.. ....	.. ..	....
II	Poor alone	Poor	Poor	Poor	Poor
III	Good alone	Good	Good	Good	Good
IV	Simultaneously good and poor	Good	Good and poor	Good and poor	Good and poor
V†	Poor and later good	Poor and good	Poor	Poor	Poor
VI†	Good and later poor	Good	Good and poor	Good	Good

* Strains used for inoculation are those listed in table 1.
† Second inoculation with alfalfa and clover 42 days after planting; with pea, 28 days; and with soybean, 30 days after planting.

strains the nodules are larger, fewer in number, and usually located on the upper portion of the root system. Although positive diagnosis of the effectiveness of a strain can not be made by the nodule size and type, a large proportion of such attempts by one familiar with the organisms and plants are successful.

The surface of the nodule was sterilized in a 1 to 500 mercuric chloride solution in order to remove the possibility of obtaining cultures from the sand rather than from the inside of the nodule. Not less than two colonies from each nodule were selected for study and identification, and wherever differences in colony characters were found several colonies were selected. In no instance was more than one strain found in any one nodule.

The results of this study show that a single individual of each of the host plants may under appropriate conditions of inoculation bear nodules formed both by effective and by ineffective strains of Rhizobia. Simultaneous inoculation with the two contrasting strains at the time of planting served to establish

both strains on the host plant, with clover, pea, and soybean, but not with alfalfa.

Although simultaneous inoculation of alfalfa at planting with the two strains of Rhizobia failed to give plants infected with both strains, inoculation with the effective strain 42 days after planting seed which had been inoculated with the ineffective strain, series V, gave plants bearing nodules formed by each strain. The reciprocal experiment, series VI, in which the good strain was used first, resulted in infection only with the good strain.

With clover, series IV, simultaneous inoculation with the good and poor strains, and series VI, inoculation first with the good strain and later with the poor strain gave infection with both strains of Rhizobia. In series V, where the poor strain was used for the primary inoculation and the good strain applied 42 days later, only the poor strain was recovered from the nodules.

With pea and soybean, only series IV, simultaneous inoculation with the two strains, served to give plants infected with the two strains. Pea and soybean are both annual plants, with a much shorter growth period than clover and alfalfa. It is possible that double infection might have been obtained in series V and VI had the second inoculation been applied at an earlier date.

Because of the realization that the failure to recover both strains from all plants to which the two strains were added might have been due to the element of chance in picking the nodules for study, another experiment was made in which a much larger number of nodules were studied. Clover was inoculated as before at planting with the ineffective strain and after 56 days a heavy inoculum of the effective strain was added to the jar. The plants were washed out when 174 days old and 100 nodules, comprising all the nodules on four of the side roots, were plated. The results here were the same as in the previous experiment—only the ineffective strain was recovered. It is characteristic of the ineffective strains to produce nodules well distributed over the root system, and many of the nodules plated in this study were located on roots which grew after the time of the second inoculation. Yet, only the ineffective strain was recovered from the 100 nodules studied.

Many other experiments will be necessary, using other strains of the organism and different methods of inoculation, before a complete analysis of the relation between the host and the lower organism can be given. Certain indications from these experiments, however, are worth noting. First, there is evidence, with clover, pea, and soybean, that the resistance of infected plants toward additional infection is greater against a new strain than against the one already within the plant. Second, the indications are that the infective power of the strains may vary independently of their effectiveness. With *Rhizobium meliloti*, the good strain, 100, is apparently more infective than the poor strain, 101, since strain 100 was recovered from all cases of double inoculation whereas strain 101 was recovered from series V only where it alone was present during the first 42 days of plant growth. Just the reverse is seen with *Rhizobium trifolii* where the poor strain, 202, appears to have greater infective power

than the good strain, 205. Here both strains were recovered where the good strain was applied first and later followed by the poor strain, but the good strain was not recovered when it was applied as the secondary inoculation.

Both of the foregoing hypotheses are dependent upon the assumption that the two strains of the organism were in contact with the root systems of the plant. An effort to accomplish this was made by the use of a very heavy inoculum which was immediately washed into the dry sand with a large volume of water. It is possible, but hardly probable, that antagonism between the two strains may have resulted in the rapid elimination of one of them.

EFFECT ON THE HOST OF DOUBLE INFECTION WITH GOOD AND POOR STRAINS OF
RHIZOBIA

The data reported in the first part of this paper have demonstrated that under suitable conditions a leguminous plant may bear, simultaneously,

TABLE 3

Effect on Pisum sativum of double infection with good and poor strains of Rhizobium leguminosarum*

SERIES	INOCULATION PLAN	STRAIN RECOVERED	DRY WEIGHTS	NITROGEN	
			gm.	*mgm.*	*per cent*
I	Uninoculated controls	. . ..	6 06	84 8	1.40
II	Poor	Poor	6 15	105 1	1.71
III	Good	Good	8 20	181.2	2.21
IV	Simultaneously poor and good	Poor and good	7.05	158 6	2.25
V	Poor, later good	Poor only	5 95	104 1	1.75
VI	Good, later poor	Good only	7 83	182 4	2 33

* The poor strain used was 310 and the good strain used was 313. Twelve plants in each series. Planted March 16. Harvested May 3. Second inoculation—28 days after planting.

nodules formed by effective and by ineffective strains of the nodule organism. The following experiments were designed to study the response of the host plant to such double infection.

Preliminary pea experiment

Dry weight and nitrogen contents are reported in table 3 for one of the experiments on peas from the preceding section. In this experiment, both the effective and the ineffective strains of the nodule organism were recovered from the plants which had been simultaneously inoculated with the two strains at planting time. The growth of the plants and the nitrogen content in this series were intermediate between that of the plants inoculated with the good strain alone and those inoculated with the poor strain alone.

When either the good or poor strain was applied first and followed after 28 days with the other, only the strain first applied was recovered. In both of these series, the plant growth and the percentage of nitrogen gave no evidence of any effect from the second inoculation.

Effect of time of inoculation

In the preceding experiment with peas, only the series in which the plants were inoculated at planting time with both strains bore nodules formed by the two strains. The failure to obtain entrance of the strain used for a second inoculation in series V and VI might have been due to the age of the plants at the time of the second inoculation. To test this point and to study further the response of plants to double infection with good and poor strains of the nodule organism, a set of experiments was started varying the time of inoculation and harvest.

In these experiments both pea (*Pisum sativum*) and red clover (*Trifolium pratense*) were used. The technic of manipulation was the same as that used in the earlier experiments except that all plants were grown in the half-gallon jars of sterilized sand. All possible precautions were taken to prevent contamination with other strains of Rhizobia.

At the end of approximately the first third of the growth period and again after about one-half of the growth period, part of the uninoculated controls and part of the plants nodulated with the poor strain, were inoculated with the good strain. The experiments were so arranged that part of the pots were washed out for analysis, (a) at the end of the first period, (b) at the end of the second period, and (c) at the completion of the experiment. Details of the inoculation are given as follows:

BEGINNING	END OF 1ST PERIOD	END OF SECOND PERIOD	END OF EXPERIMENT
Inoculation	Inoculation	Inoculation	
Controls	H*		
Controls		H	
Controls			H
Controls	Good	H	
Controls	Good		H
Controls		Good	H
Poor	H		
Poor		H	
Poor			H
Poor	Good	H	
Poor	Good		H
Poor .		Good	H
Good	H		
Good .		H	
Good...			H

* The H indicates the period at which each set was harvested   Triplicate pots were carried in each case

The data obtained in these experiments are recorded in table 4. The plants harvested at the end of the first period, with either red clover or peas, showed no effect of the inoculation although nodules were present on the roots. Evidently the seed nitrogen was adequate to carry the plants this far. At the end of the second period, the plants from seed which had been inoculated with the good strain at planting were much better than any of the others, with both peas and red clover. The corresponding sets inoculated with the poor strain had made

TABLE 4

Effect on the host plant of double infection with good and poor strains of Rhizobia

INOCULATION	ALASKA PEA*			RED CLOVER†		
	Dry weight	Nitrogen		Dry weight	Nitrogen	
	gm	*mgm*	*per cent*	*gm*	*mgm*	*per cent*
Harvest at the end of the first period						
Control	2 12	68 8	3 24	0 23		
Poor strain	2 17	65 6	3 02	0 16		
Good strain	2 03	58 7	2 89	0 21		
Harvest at the end of the second period						
Control .	4 76	85 2	1 79	0 71	7 5	1 05
Control plus good	4 30	82 1	1 91	0 62	8 4	1 34
Poor plus good	4 85	89 7	1 85	0 53	8 1	1 51
Poor	4 76	89 1	1 87	0 70	10 0	1 43
Good	5 51	166 9	3 03	1 47	38 7	2 57
Harvest at the end of experiment						
Control	5 85	95 9	1 64	0 81	12 6	1 55
Control plus good, early	8 36	211 5	2 53	5 54	110 3	1 99
Control plus good, late	4 88	61 9	1 27	2 67	55 6	2 08
Poor plus good, early	6 40	106 2	1 66	3 10	58 2	1 88
Poor plus good, late	7 13	112 6	1 58	2 91	53 9	1 85
Poor	5 93	97 8	1 65	1 16	24 0	2 06
Good	15 09	344 2	2 28	8 71	176 5	2 02

* Alaska pea inoculated with good strain, 313, and poor strain, 310   Plants 19 days old at end of first period, 31 days old at end of second, and 52 days old at end of experiment

† Red clover inoculated with good strain, 205, and poor strain, 202, plants 38 days old at end of first period, 61 days old at end of second, and 123 days old at end of experiment

essentially the same growth as the controls. No effect was apparent from the secondary inoculation with the good strain, either of control plants or plants already carrying the poor strain.

At the end of the experiment, however, definite differences were apparent in the growth and appearance of the various series. The dry weights and nitrogen contents of these plants are displayed graphically in figures 1 and 2, and plate 1 shows a photograph of the clover series at this time. With both clover

and peas, the plants from seed inoculated at planting time with the good strain were much the best, in total plant growth and in the amount of nitrogen in the plant tissue. With peas, the plants from seed inoculated with the poor strain at planting were essentially like the controls. With red clover, however, the series inoculated with the poor strain was slightly better than the control series.

Delayed inoculation with the good strain on previously uninoculated plants served to increase markedly both plant growth and nitrogen content with each host species. The early inoculation was much more effective than the late
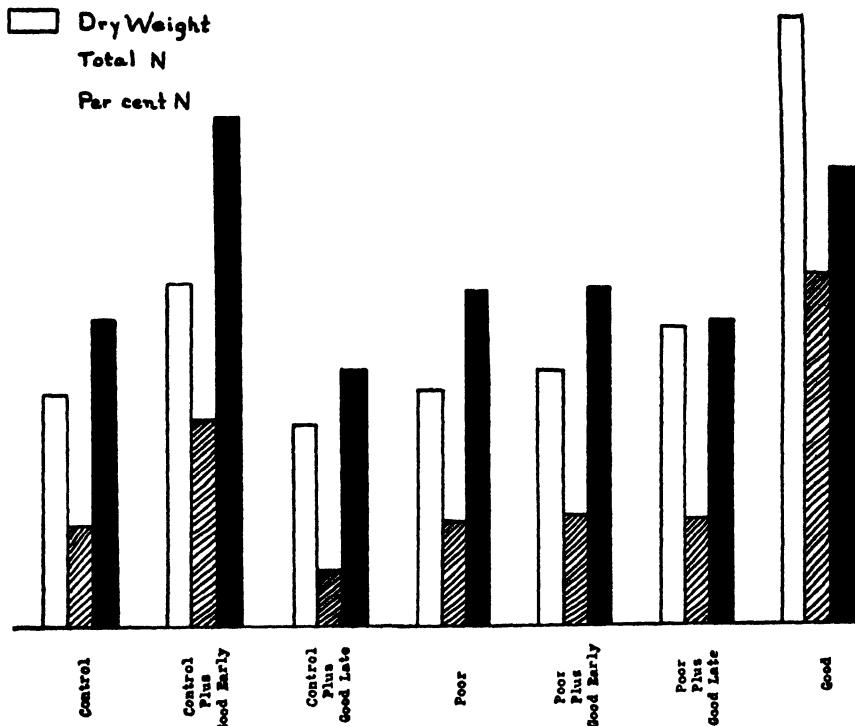

FIG. 1. THE EFFECT OF DOUBLE INFECTION WITH GOOD AND POOR STRAINS OF *Rhizobium leguminosarum* ON THE DRY WEIGHTS AND NITROGEN CONTENT OF PEAS
(*Pisum sativum*)

inoculation in each case. Quite a different picture was presented where a secondary inoculation with the good strain was applied to plants already carrying the poor strain. The effect of the good strain in stimulating plant growth here was much less marked than where the good strain was applied under similar conditions to nodule-free plants. Also the time of applying the good strain seemed to be of less importance, as the plants receiving the late inoculation with the good strain finished the growth period with essentially the same dry weights and nitrogen contents as those receiving the earlier inoculation. This substantiates the finding of the isolation experiments; plants which

are nodule-free are much more easily infected with a good strain of Rhizobia than are plants which are already infected with a poor strain of the organism.

With peas, the dry weights of the plants at harvest are roughly proportional to the percentage of nitrogen. With red clover, however, there is little variation in the percentage of nitrogen in the plants of the different series, although the dry weights are quite different.

Comparison of natural versus artificial inoculation

The previous experiments have demonstrated that the earlier the good strain gained entrance into the plant the more the plant profited from the association.
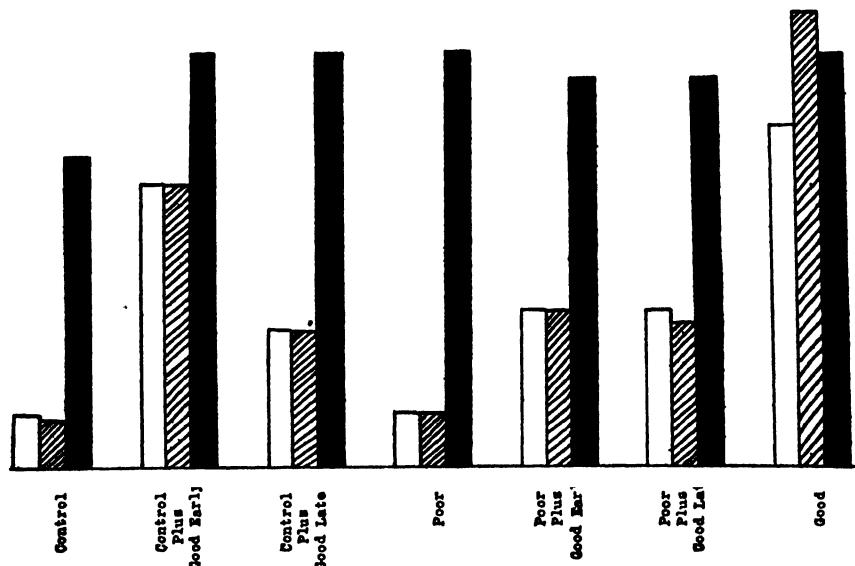
Fig. 2. The Effect of Double Infection with Good and Poor Strains of *Rhizobium trifolii* on the Dry Weights and Nitrogen Content of Red Clover
(*Trifolium pratense*)

This was particularly true in cases in which both organisms were present. Under field conditions either the good or poor strain may be present in soil in which inoculated seed is sown. Also experience with commercial cultures has demonstrated that not only good but also poor strains are at times used for seed inoculation. The following experiments were set up to determine the value of inoculating seed with an effective strain of the organisms and then planting it in soil carrying an ineffective strain; also to determine the effect of planting,

in a soil which carries an effective strain of the organisms, seed which had been inoculated with an ineffective strain.

In the first of these experiments, with peas and soybeans, sterile sand in half-gallon pots was inoculated with either the good or poor strain by adding 1 cc. of a heavy bacterial suspension of the organism and then bringing up to optimum moisture with sterile water. These inoculated pots were set aside for 3 days before planting. The seed were first rendered bacteria-free and then inoculated with the appropriate strains of the nodule organism. The inoculation plan and the data obtained from these experiments are presented in table 5.

With soybean when only one strain was used, essentially the same results were obtained regardless of whether the seed or the sand received the inoculum. With either host plant, those inoculated with the ineffective strain gave dry

TABLE 5

Comparison of soil versus seed inoculation of soybeans and peas with good and poor strains of Rhizobia

INOCULATION	SOYBEAN*			PEA†		
	Dry weight	Nitrogen		Dry weight	Nitrogen	
	gm	mgm	per cent	gm	mgm	per cent
Control	9 6	114 2	1 18	3 9	126 7	3 25
Sand, good strain	12 9	227 2	1 76			
Sand, poor strain	9 2	107 6	1 17			
Seed, good strain	11 5	206 2	1 79	5 1	175 7	3 44
Seed, poor strain	9 7	115 4	1 19	4 3	133 0	3 08
Sand, poor strain, seed, good strain	10 9	205 2	1 88	4 8	170 6	3 49
Sand, good strain, seed, poor strain	12 5	250 5	1 95	5 2	176 5	3 35

* Soybean inoculated with good strain, 501, and poor strain, 507. Planted May 21 Harvested July 6   Three pots with four plants each

† Pea inoculated with good strain, 313, and poor strain 310   Planted September 26 Harvested November 23   Three pots with four plants each.

weights and nitrogen percentages which were approximately the same as the control plants. Inoculation of plants with the effective strain markedly increased the plant growth and nitrogen content of the plants.

Under the conditions of these experiments with either host, the effective strain seemed to dominate over the ineffective strain, regardless of which was applied to the seed and which was present in the soil. It was noticeable, however, that the strain which was in the sand seemed to be slightly more active. This was probably due to the larger numbers present in the sand. Where the sand carried the good strain and the seed the poor strain, the plants made just as good growth as the plants in those pots containing only the good strain. Slightly poorer plant growth resulted from the planting of seed inoculated with the good strain in sand carrying the poor strain.

In these experiments, conditions as they might exist in the field were only

approximated, since the inoculum applied to the sand was much heavier than would be found under ordinary soil conditions. To approach normal soil conditions more nearly, a second experiment was started, in which the sand inoculation was that residual from a first crop.

A number of pots of sterilized sand were planted with peas (*Pisum sativum*), part of which had been inoculated with an effective strain, 313, and part with an ineffective strain, 310. At maturity the tops of these plants were harvested and the pots with undisturbed root systems were thoroughly moistened with sterile water, covered with sterile paper, and set aside for 3 weeks. Although sterile sand was used at the start, exposure to the greenhouse air resulted in the

TABLE 6

Comparison of natural versus seed inoculation of pea (Pisum sativum) with good and poor strains of Rhizobia

STRAIN OF FIRST PLANTING[*]	RE-INOCU-LATION, SECOND PLANTING[*]	COLOR	NODULES	DRY WEIGHT	NITROGEN	
First crop—tops only						
				gm.	*mgm.*	*per cent*
Poor	. . . . .	Pale green	Small scattered	4 05	76.9	1.90
Good	. . . . .	Rich green	Large on tap root	6 30	178 9	2.84
Second crop—tops and roots						
Poor	0	Pale, stunted	Many small	4 49	134 7	3 00
Poor	Poor	Pale	Small and medium, scattered	4 86	148 7	3.06
Poor	Good	Good green	Small and medium, of both types	5 08	160 5	3 16
Good	0	Good green	Medium to large	6 01	204 3	3 40
Good	Poor	Medium green	Medium to small	4 48	147 8	3.30
Good	Good	Good green	Medium to large, good type	6 02	201.6	3 35

* Pots in triplicate with 6 plants in a pot—18 plants. Three-week interval between the harvest of the first crop and the planting of the second.

entry of many types of organisms and the roots partially decayed in the 3-week rest period. These plants had been grown in a separate greenhouse and control plants did not show nodules, indicating that little if any contamination of the pots with nodule organisms had occurred. For the second planting, bacteria-free seed were inoculated either with the good or poor strain and planted in these pots.

The inoculation plan and the data obtained were presented in table 6. Seed reinoculated with the homologous strain, i.e., the strain already present in the pots, gave essentially the same plant growth as did seed that was not inoculated. Inoculation of seed with the heterologous strain, i.e., the contrasting strain, however, gave marked results. When planted in the sand carrying the

poor strain, seed inoculated with the good strain gave improved plant growth in comparison with either uninoculated seed or seed inoculated with the poor strain. On the other hand, with the sand carrying the good strain, inoculation of the seed with the poor strain reduced plant growth in comparison with either uninoculated seed or seed carrying the good strain.

It is interesting to note that all of the plants in the second planting bore many nodules, and that the type of nodules corresponded well with the plant growth. In the sand carrying the poor strain, only the plants from seed inoculated with the good strain bore large nodules. All the others bore many small nodules. In the other series only the plants from seed inoculated with the ineffective strain bore the numerous scattered nodules characteristic of the ineffective strain, whereas the others had fewer but larger nodules.

Under these conditions which more nearly approximate field conditions, it is apparent that seed inoculation with an effective strain may materially improve plant growth where the soil already contains many nodule-forming organisms of an ineffective type. Also, inoculation of seed with an ineffective strain of the organism may be detrimental to crop growth if the soil already contains an effective strain of the organism.

SUMMARY

Isolation studies from leguminous plants, clover, alfalfa, pea, and soybean, inoculated with both effective and ineffective strains of the nodule organisms show that a single plant may bear at one time nodules formed by each of the strains. In no instance were two strains found in a single nodule.

The evidence from these studies indicates that the strains of the nodule organism may vary in their infective power independently of their ability to aid the growth of the host plant, since certain strains seemed to be able to enter the plant more readily than others.

Plants already infected with one strain of the nodule organism resist the entrance of a contrasting strain to a much greater extent than do nodule-free plants. On the other hand, nodulated plants are apparently open to further infection by the homologous strain of the nodule organism as long as new roots are formed. This is particularly true of plants bearing the ineffective strain, where the nitrogen supply of the plant is deficient

Plants bearing both an effective and an ineffective strain of the nodule organism give growth and nitrogen fixation intermediate between those bearing either strain alone.

The earlier the effective strain gains entrance into the plant the more benefit the plant derives from the association. This effect is more pronounced with plants which were previously nodule-free than with plants already infected by the poor strain.

Improved plant growth results from inoculation of seed with an effective strain when it is sown in soil carrying an ineffective strain. On the other hand, inoculation of seed with an ineffective strain is detrimental to plant growth, if the seed is planted on soil already carrying an effective strain of the organism.

The results of these studies indicate the necessity of using for seed inoculation only effective strains of the nodule organism, since definite detrimental results may occur from the use of ineffective strains.

It is probable that many of the favorable results obtained from seed inoculation on old land are due to the introduction of more effective strains of the nodule organism. Also that many of the failures to produce increased plant growth by seed inoculation are due to the use of ineffective strains of the organism.

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PLATE 1

RED CLOVER PLANTS JUST BEFORE HARVEST (123 DAYS OLD) SHOWING THE GROWTH FOLLOWING INOCULATION AT VARIOUS TIMES

D. H. DUNHAM AND I. L. BALDWIN
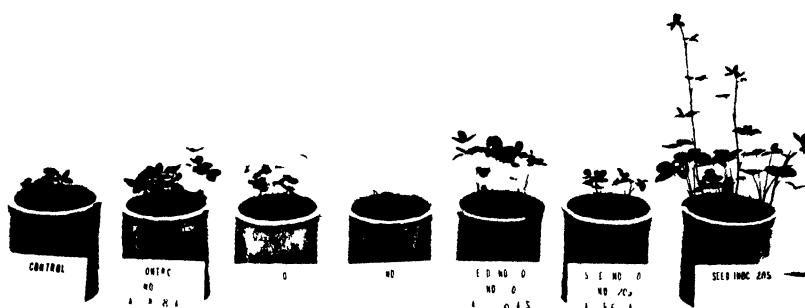
THE FIXATION OF NITROGEN BY LEGUMINOUS PLANTS UNDER BACTERIOLOGICALLY CONTROLLED CONDITIONS[1]

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A study of the biochemistry of nitrogen fixation by the Leguminosae has been made possible by a grant from the Herman Frasch fund. This problem is characterized by a certain phenomenon, viz., the fixation of elemental nitrogen as a result of an association between certain bacteria and plants. This association effects a result which apparently neither member can produce alone. It is patent that any attack upon the question of what actually obtains in the fixation must have as a keystone, studies of the communal activities of the plant and bacteria. The history of scientific inquiry, however, teaches that a comprehension of the interaction of several components in a complex system is best achieved by analytical means. That is, a knowledge of the properties of each variable when isolated is always an aid, and frequently a necessity, for a successful attack on the complicated relationships existing in the system as a whole. Obviously, experiments directed toward an understanding of the processes involved in nitrogen fixation by the Leguminosae must be separated into two groups: (*a*) those concerned with the isolated components, particularly the bacterium, and (*b*) those concerned with the association of plant and microörganism. This partition of the research may give rise to investigations that apparently are only superficially connected. Thus a knowledge of the physiology of the causal organism is certainly germane to a complete understanding of the mechanism of the fixation, but studies in this direction possibly will lend a certain diffuseness to a series of researches that purport to deal only with the biochemistry of nitrogen fixation by the Leguminosae. However, as the work progresses, the relationship of the separate phases to the general project should become apparent and the desired unity achieved.

In this paper are reported results of experiments that deal with the fundamental problem of fixation by the host plant and bacteria. Considerations, to be discussed in detail later, indicated the desirability of an investigation of the behavior of the plant with the proper bacteria present under conditions which exclude the complications induced by the presence of other microör-

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[1] Herman Frasch Foundation Research in Agricultural Chemistry, paper no. 17. Contribution from the departments of agricultural chemistry and agricultural bacteriology, University of Wisconsin.

ganisms. In many questions concerned with nitrogen fixation by leguminous plants, it is neither necessary nor desirable that the experiments be carried out under conditions which prevent the growth of foreign bacteria. If the results are to be applied to practical agriculture, the conditions of the experiments should simulate as closely as possible those prevailing in the field. But for problems of a more theoretical nature, it is indispensible that our attention be restricted to the most simple system possible, in order that the essential factors may be separated from the incidental ones. Specifically, we wish to ascertain whether nitrogen is fixed by legumes when only members of the Rhizobium species are present, and if so, the influence of other organisms on the fixation. A second problem considered in this paper is that which deals with the variability of nitrogen fixation by legumes inoculated with different strains of the proper organism, another instance in which freedom from contaminating forms is of utmost importance.

<div align="center">LITERATURE</div>

The method used to grow the plants under bacteriologically controlled conditions has been described in a previous paper (14). Essentially it involved the growth of small seeded leguminous plants on an agar substrate in 12- and 32-ounce bottles protected from air contamination with cotton plugs. Once the method for growing plants free from microörganisms was selected, the question arose:—Will the conditions adopted as necessary to insure complete freedom from bacteria allow nitrogen to be fixed by the association of bacteria and plant? Therefore, our first experiments were devoted to the question of nitrogen fixation in agar.

The fixation of nitrogen under conditions which keep the plant roots submerged is disputed. Hiltner (11) states that although alder plants form nodules in water culture, the plants derive no benefit from the nodules. Nobbe and Hiltner (19) found that wild acacia attained better growth in water cultures when some of the nodules were exposed to air. Golding (6) attempted to verify this finding with peas. He found that a slight fixation of nitrogen took place in water cultures with the roots of the plants covered. Plants grown in water cultures, the surface of which was covered with oil, fixed no nitrogen. The fixation of nitrogen by plants with exposed roots, or with roots covered, but with air or oxygen passing through, was no greater than that fixed by plants, the roots of which were submerged. Harrison and Barlow (9) used a maltose-wood ash-agar substrate for growth of legumes and observed nodule formation on garden bean (*Phaseolus vulgaris*), hairy vetch (*Vicia villosa*), pea (*Pisum sativum*), and soybean (*Soja Max*). They reported that uninoculated plants became yellow and gradually withered as if from nitrogen starvation, whereas the inoculated plants thrived for as long as 5 months. Photographs of vetch (5 months) inoculated and uninoculated exhibited unmistakable evidence of benefit through inoculation, but peas 2½ months old showed very little benefit. Very likely this was due to the large food reserve

in the pea seed.   There is no doubt that these experiments demonstrated the fixation of nitrogen in an agar substrate, but the actual quantities fixed are not given.

The only quantitative data from experiments in which agar was used are those given by Thornton (20). This worker claims that inoculated alfalfa grown in wide test tubes fixed nitrogen only when the nodules had access to air. Unfortunately the data in support of this claim are too meager to allow adequate judgment.   Five tubes in which the nodules were exposed to the air had a mean total nitrogen content (agar plus plants) of 3.53 mgm.; four tubes in which the nodules were imbedded in the agar had a mean of 1.88 mgm.; the single control contained 2.26 mgm. of nitrogen.   The nitrogen content of the tubes in which the nodules were exposed to the air was extremely variable, ranging from 2.63 to 4.22 mgm.; the amount fixed on the basis of the single control (the variability of which is of course not calculable) was 1.27 ± 0.330 mgm.   The variability of the four tubes in which the nodules were imbedded in the agar was much less; the range was 1.55 to 2.06 mgm., but a nitrogen loss of 0.38 ± 0.11 mgm. was sustained.   Both the gain and the loss of nitrogen are significant from a statistical point of view, and any theory based on these data should explain the loss suffered by the plants whose nodules were imbedded. The explanation in all likelihood lies in the single control; if more controls had been included, it is probable that the loss noted would have been within experimental error.   It might be argued that data of so few samples cannot be treated in a statistical manner, but in that case sufficient samples should be taken to allow for the variability.   The evidence presented by Thornton's photographs is more conclusive.   The plants in which the agar is intact differ little from the controls, while those in which the agar has shrunk away from the sides of the test tubes show a marked improvement.   Whether this shrinkage actually exposed the nodules to the air cannot be decided from the photographic data.

A second reason for the investigation of fixation in agar under bacteriologically controlled conditions was to shed light on the question of whether members of the root nodule bacteria are the sole organisms concerned in the fixation of atmospheric nitrogen by legumes.   Observations tending to make some workers skeptical that this fundamental problem has been completely solved are: (a) reports of the presence of organisms other than rhizobia in nodules; (b) the difficulty of growing plants in the complete absence of contaminators from soil and air; and (c) the negative results obtained by numerous experimenters in regard to nitrogen fixation by rhizobia apart from the host plant [Hopkins (13), Löhnis (17), and Allison (1)].   It is commonly accepted that members of the genus *Rhizobium* are the sole causal organism concerned in the utilization of atmospheric nitrogen by legumes.   Nevertheless, Beijerinck after 30 years' work in this field expressed doubt as to whether this had been demonstrated under experimental conditions that eliminated all other organisms.   He says (2):

. . . it proved hitherto impossible in sand cultures to bring Leguminosae to complete development by infection with *B. radicicola* only, and with the exclusion of all other microbes. Such experiments are at the end of the vegetation period rich in various other species, in particular in *B. fluorescens liquefaciens* and the nitrogen fixing spore former, *Cl. pasteurianum* and *Helobacter cellulosae*. This observation holds good as well for those experiments made by myself as for those of others, and this should never be lost sight of when reading descriptions of the infection experiments with so-called "pure cultures." It is a well-known fact that the Papilionaceae, when cultivated in liquid, do not fix the atmospheric nitrogen indifferently whether they produce tubercles or not.

EXPERIMENTAL

Methods

The technique used for growing the plants and testing the medium for con-taminating forms has been described in a previous publication (14). Briefly, it consists in the sterilization of the seeds in a modified Dakin solution, the germination on sterile blotting paper, and the transfer of the seedling to a Crone-agar substrate in 12-ounce or 32-ounce bottles. This transfer is done in a special chamber so constructed as to eliminate contamination from the air. After growth of the plants, a test of the substrate is made for foreign organisms by making subcultures in litmus milk. In growing plants in the greenhouse, and especially in closed containers, it must be recognized that a highly artificial environment is forced upon the plant; a circumstance that must be considered in an interpretation of results. Care must be exercised to reduce as far as possible the effect of factors that are detrimental to the physiology of the plant. For example, during the months from December to March, there is a lack of sunlight in this latitude. Accordingly, artificial illumination by means of a battery of 200-watt Mazda lamps was employed during these months with apparent benefit to the growth of the plants. On the other hand, during the spring and summer months the plants must be protected from excessive illu-mination with resultant overheating. This was accomplished by placing the culture bottles in wet sand to the depth of the agar substrate; the use of fans to increase circulation of the air and enhance evaporation is also advisable. The maintainence of a proper temperature is especially important during the early stages of the plants' growth. In an experiment in which these precautions were not observed, two unusually warm days in early spring were sufficient so to retard the development of the plants that no nitrogen was fixed. Although the plants were not noticeably injured, and in spite of the fact that numerous nodules were formed, maximum development was not attained. This de-pendence of nitrogen fixation on the general well-being of the plant has been noted in all of our experiments. It appears that the quantity of nitrogen fixed is determined largely by the development of the plant instead of a de-pendence of the latter on the available nitrogen. This view received support by the inclusion of controls to which nitrate nitrogen was added. In exposure to a variety of greenhouse conditions which resulted in different degrees of development, it was found that the nitrogen fixed by the inoculated cultures

was practically identical with the nitrogen assimilated by the nitrate controls. In all cases, the latter did not assimilate the total nitrogen available. This would indicate that under the experimental conditions employed, factors other than the available nitrogen are the limiting ones in the growth of the plant. Some of these factors are illumination, humidity, and gas exchange. Insufficient illumination slows down the growth of the plant and results in a yellowish green color, indicative of chlorosis. Artificial illumination remedies, but does not entirely correct this. Unfavorable humidity conditions and inadequate gas exchange are caused by lack of air circulation through the cotton plug of the container. On warm days water collects in drops on the walls of the container and the resultant saturated atmosphere retards transpiration. Consequently the plant is not kept properly cooled and the intake of nutrients is probably decreased. Knudson (15) observed that CO_2 uptake by strong KOH solution in cylinders open to the air was three times that of cylinders closed with cotton plugs. This decrease in rate of diffusion of CO_2 would decrease carbohydrate synthesis by the plant and the latter condition would in all likelihood be reflected in the entire metabolic activity of the plants. At present, experiments are in progress in which an effort to reduce the effect of this factor by the use of loose cotton plugs covered with a thin layer of absorbent cotton impregnated with a solution of sodium benzoate (5 per cent) and a bacteriostatic dye, e.g., crystal violet. It is hoped that this method will prove efficient in preventing the entrance of microörganisms through the plug and will at the same time allow a better gas exchange. Experiments are also in progress to determine the effect of aeration on the growth of the plants.

FIXATION OF NITROGEN BY RED CLOVER UNDER STERILE CONDITIONS

The results of experiments on the fixation of nitrogen by plants grown in agar are given in table 1. A summary of 90 analyses, each analysis representing 10 to 12 plants, is presented. In order to save space, only the range of the analyses of the various samples are presented (columns 3 and 4) together with the standard deviation (column 6); this latter gives a fairly accurate idea of the "scatter" of the individual values, as in general, one-half of the values observed fell within the theoretical 0.674 times the value of ,he standard deviation (Probable error) measured from the mean or average. The standard deviation of the population was calculated from the usual formula

$$S.D. = \sqrt{\frac{S(x-\bar{x})^2}{n-1}}$$

where x is the individual values observed, $\bar{x}$ the mean or average, and n, the number of samples considered. The average of the analyses in each series is given in column 5, together with its standard deviation calculated from

$$\sigma_{mean} = \frac{S.D.}{\sqrt{n}}$$

In column 7 is given the total nitrogen fixed per 10 (or 12 plants) with the standard deviation of this value. This latter was calculated from the formula:

$$\sigma_{\text{diff}} = \sqrt{\sigma_A{}^2 + \sigma_B{}^2} \quad {}^2$$

where σ_A = standard deviation of mean of control

σ_B = standard deviation of mean of inoculated plants.

The data in experiments I and II are divided into two groups (column 8), viz., those cultures which showed the presence of contaminating organisms at the end of the experiment and those which showed only rhizobia. It is to be noted that in neither experiment was there any indication that the presence of incidental contamination—molds and bacteria—affected the nitrogen fixed. This was true also of experiment III in which the divided data are not given, because of small numbers in each class.

The first point to be observed in the results is the actual quantity of nitrogen fixed. In experiments I and II this amounted to an average of 3.5 mgm. per 10 plants. These experiments were made in the late summer and fall. Experiment III was made from November 10 to February 22, and artificial light was used to supplement the daylight. The growth was slower, but the plants were stronger, had better color, and in general were much more healthy-appear-

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[2] It should be recognized that the standard deviation calculated by this formula is only approximate and in this case is very likely a maximum value. The true formula for the standard deviation of the difference of two means is

$$\sigma_{\text{diff}} = \sqrt{\sigma_a{}^2 + \sigma_b{}^2 - 2r\,\sigma_a\,\sigma_b}$$

where r = correlation coefficient between A and B and the other symbols have the usual significance.

Lacking knowledge of the value of r it is usually assumed to be zero, i.e., the values of means are independent of each other and the formula reduces to the one given in the text. In the case under discussion, the value of r is probably positive, since experience has indicated that the larger seeds of a plant like clover, viz., one with limited food reserve, give a better initial growth, which enables the plant to fix more nitrogen. That is, the higher the mean of the control, the higher the mean of the inoculated plant. This positive value of r would lower the standard deviation of the difference, which would increase the probability of the difference being significant   In this particular series of experiments, correction of the σ_{diff}. would not increase the certainty of the conclusions arrived at, since all of the differences noted are 10 or more times their standard deviation. However, it is well not to lose sight of the fact that the formula in the text involves the assumption that the means are independent. In the comparison of samples inoculated with different organisms, it is probable that the correlation coefficient between the mean is high and positive, since the same environmental condition would tend to affect the plants alike, independently of the organisms used. As already pointed out, the nitrogen fixed appears to be largely dependent on the growth made by the plant; therefore the assumption that r is $+ 1$ rather than zero would probably be nearer the truth. However, in the absence of an exact determination of the value of r it is advisable to use a value which would tend to minimize the significance of the difference noted rather than to exaggerate the significance of the data. Therefore, in our experiments zero was used for the value of r; if there are any reasons to suspect that r might be negative, it is advisable to assume $r = - 1$ and then use the complete formula.

ing plants. The nitrogen gained averaged 11.57 mgm. per ten plants. This was the maximum figure in any of our experiments. Experiment I was made in 32-ounce round bottles (10 plants to a bottle); experiment II, in 12-ounce bottles (6 plants to a bottle); experiment III, in round bottles and large, tall test tubes. The latter were especially satisfactory, as the increased space obviated to a certain extent the poor gas exchange. Although the absolute quantity of nitrogen fixed was not imposing, a comparison made with that

TABLE 1

Fixation of nitrogen by red clover under sterile conditions

TREATMENT	NUMBER OF SAMPLES	NITROGEN PER 10 PLANTS				NITROGEN FIXED PER 10 PLANTS	REMARKS
		Min mum	Maximum	Average	Standard deviation		
		mgm	*mgm*	*mgm*	*mgm*	*mgm*	
Experiment I, 70 days							
Controls	8	1 8	2 8	2 23±0 12	0 33		. . .
Culture 200	10	4 9	7 5	5 85±0 26	0 82	3 62±0 28	Sterile
	6	4 7	6 4	5 90±0 28	0 69	3 67±0 30	Contaminated
Experiment II, 51 days*							
Controls	11	1 6	2 8	2 35±0 12	0 37		Sterile
	11	1 8	3 0	2 12±0 11	0 34		Contaminated
Culture 200	9	4 7	6 7	5 62±0 24	0 72	3 27±0 27	Sterile
	12	4 0	7 4	5 60±0 31	1 08	3 48±0 33	Contaminated
Experiment III,† 105 days							
Controls	10	1 8	2 8	2 25±0 09	0 28		3 sterile 7 contaminated

* Twelve plants in this experiment
† Controls began to turn yellow at 45 days and were analyzed at 78 days

originally in the seed shows that in experiments I and II the quantity of nitrogen more than doubled, whereas in experiment III it was increased fivefold. In all cases there is no question about the significance of the quantity fixed, as shown by column 6. Plate 1, figure 1, shows the influence of inoculation on clover grown in agar, and here also there is no mistaking the effect of inoculation.

That the quantity of nitrogen fixed is sufficient for the requirements of the

plants was indicated by an experiment in which, in addition to the usual un-inoculated controls, 14 uninoculated controls were included to which 10 mgm. of nitrogen as KNO_3 was added to the 100 cc. of agar in each bottle. After 3 months the plants were analyzed. Both the inoculated plants and those supplied with KNO_3 were green and healthy with little difference in appearance, but the plants which were neither inoculated nor supplied with nitrate were yellow and withering. Analyses gave the following results: controls, 1.38 ± 0.04 mgm.; controls plus nitrate, 3.99 ± 0.06 mgm.; inoculated with culture 200, 3.46 ± 0.06 mgm. These results are taken to mean that the limit of nitrogen fixed in agar is a function of the growth of the plants rather than the reverse. Even though plants were plentifully supplied with nitrate nitrogen, they assimilated only about 25 per cent of that available, and their growth was no better than that of plants which obtained their nitrogen from the air.

Finally, in these experiments and in many others discussed later, no evidence was obtained that exposure of the nodule to the air is a prerequisite for assimilation of atmospheric nitrogen. The agar used was usually 0.8 per cent, just sufficient to form a firm gel; there was no indication of shrinkage from the sides of the bottle or of cracks in the body of the agar. The discrepancy of these results with those of Thornton are not easily explained. It is possible that because of the larger agar surface exposed in the containers used in our experiments, a better opportunity was available to carry on whatever gas exchange is required by the roots. Thornton does not state the diameter of the tubes used for the growth of the two alfalfa plants. From the photograph, they appear to be the 22 x 200 mm. size. A comparison of the surface exposed in these types of containers per volume of substrate is as follows:

	Total surface	*Surface per cc*
Test tubes	400 mm ^{2}	17 5 mm ^{2}
12-oz bottle	2,400 mm ^{2}	24 mm ^{2}
32-oz bottle	5,700 mm ^{2}	22 8 mm ^{2}

From the foregoing figures it is evident that there was more surface in the large container per unit volume of substrate than in the test tubes. This would allow the equilibrium between dissolved gases and the atmosphere to adjust itself more rapidly and favor saturation of the agar with nitrogen and oxygen in the larger containers. Also, this effect is increased, because the volume of substrate-per plant was higher in our experiments. Thornton used two plants per 22.5 cc. (2½ inches in test tube) or 11.25 cc. substrate per plant. In the 12-ounce bottles 16.67 cc. substrate were available per plant and in the 32-ounce bottle 25 cc. per plant. It follows that if in these larger containers there was better opportunity for gas equilibrium between the substrate and the atmosphere and there was a greater volume of substrate for each plant to use as a source of gases, this would tend to allow the plants to obtain needed gases by diffusion processes rather than by actual exposure of roots. The importance

of oxygen is not disputed in this connection, but the necessity of the plants' obtaining nitrogen through the roots makes it imperative that we do not overlook the latter gas in our interpretation.

THE EFFECT OF OTHER MICROÖRGANISMS

As noted in table 1, the presence of contaminating forms did not affect the amount of nitrogen fixed. In these experiments, however, the contaminants were accidental, and the question of whether certain organisms accompanying rhizobia in nature might aid in the fixation could not be decided on the basis
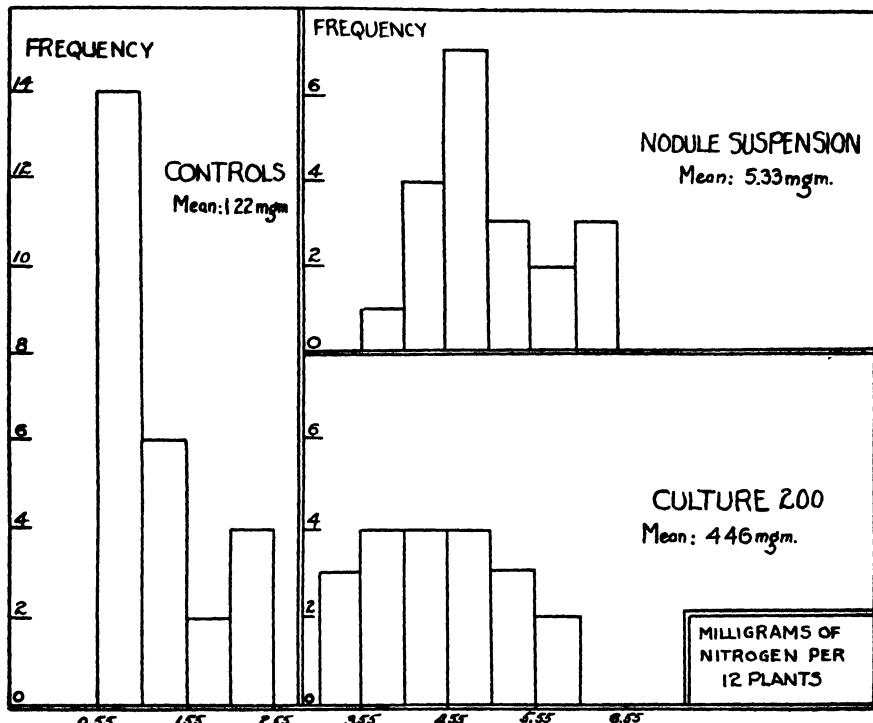
FIG. 1. FIXATION OF NITROGEN IN STERILE AGAR CULTURES (EXPERIMENT IV)

of the data given in that table. In an effort to settle this question, a number of experiments were made which would allow the presence of organisms likely to be associated with the root nodule bacteria. This was done by the comparison of nitrogen fixation by sterilized and unsterilized seeds in agar substrate and by the use of a nodule suspension in comparison with pure culture inoculum. In some cases the outsides of the nodules were sterilized by the same method as was used to sterilize the seed, whereas in others a suspension was made without this treatment. It is doubtful whether the surface sterilization actually removed all of the contaminants on the outside of the nodule, but at

least partial sterilization was effected. The results of these experiments are given in figures 1 and 2 and in table 2. In experiment IV, the data of which are given in the plates, the fixation was quite satisfactory, but in experiment V (table 2) the nitrogen fixed was only 1 to 1½ mgm. per 10 plants. Experiment V was made during the winter, and the growth of the plants was poor, hence it is probable that fixation was so repressed that differences due to treatment did not have an opportunity to manifest themselves. The data given in figures 1 and 2 show that fixation was much better in the case of the non-sterilized seeds. There was also evidence that nodule suspensions resulted in
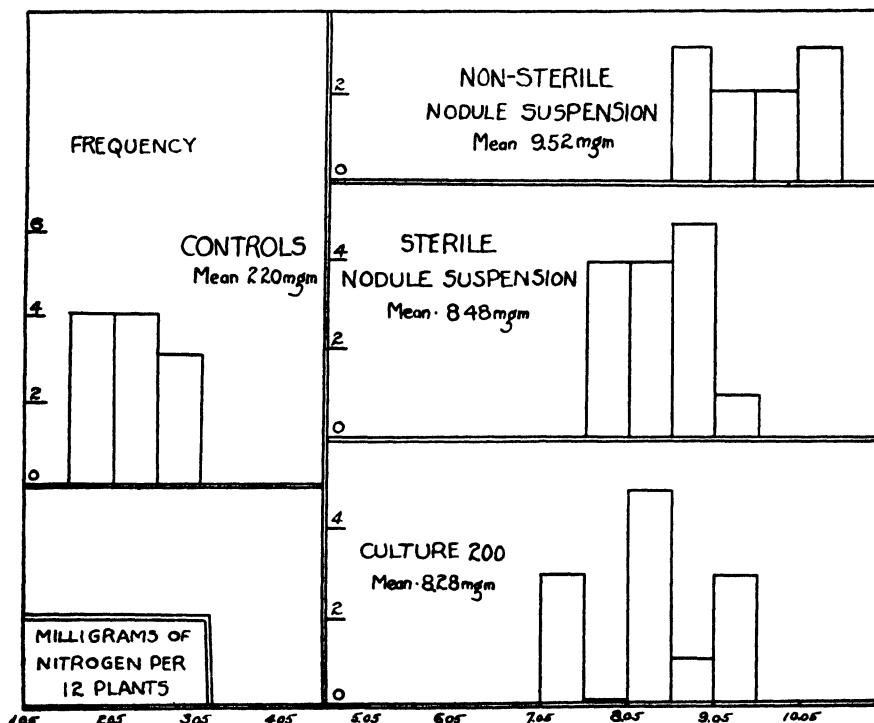
FIG 2 FIXATION OF NITROGEN IN NON-STERILE AGAR CULTURES (EXPERIMENT IV)

larger fixation than did the pure culture. An interpretation of these data must be made with utmost caution. In view of the evidence that the quantity of nitrogen fixed is dependent on the general growth of the plant, an explanation of changes in the latter would suffice for the observations made. It is quite possible that the process of sterilization so injures the seed that it is not capable of as vigorous growth as is the unsterilized seed. Some evidence of the effect of sterilization was obtained by a comparison of total nitrogen before and after sterilization. It was found that 16.4 per cent of the nitrogen in the seed was lost in the process. The loss of this soluble nitrogen together with

other food reserves probably retards the development of the seedlings and may result in a set back that is never completely overcome. A second factor that is possibly concerned in the increased fixation noted with the unsterilized seed is the CO_2 made available by the activities of contaminating organisms. If the synthetic processes of the plants are limited by the quantities of CO_2 that diffuse through the cotton stoppers, any agent that increases the CO_2 in the culture bottle would cause an increased plant growth and, hence, nitrogen fixed. The beneficial effect of contaminating microörganisms would then be explained on the basis of CO_2 produced in their respiration.

In an effort to increase CO_2 inside the culture bottles, a few plant cultures were set up under sterile conditions with 0.1 per cent glucose added to the agar. It was expected that rhizobium or any incidental contamination would grow

TABLE 2

Comparison of fixation by red clover under sterile and non-sterile conditions (Experiment V)

TREATMENT	NUMBER OF SAMPLES	NITROGEN PER 12 PLANTS				NITROGEN FIXED PER 12 PLANTS	STERILITY*
		Minimum	Maximum	Average	Standard deviation		
		mgm	mgm	mgm	mgm	mgm	per cent
Sterilized seeds, 60 days							
Control	10	1 4	1 7	1 53±0 045	0 13		86
Culture 200	10	2 3	3 1	2 67±0 073	0 22	1 14±0 086	100
Nodule suspension	10	2 7	3 6	3 09±0 086	0 26	1 56±0 097	10
Unsterilized seeds, 60 days							
Control	10	1 7	2 1	1 97±0 062	0 19		0
Culture 200	12	1 9	3 4	2 73±0 141	0 49	0 76±0 154	8
Nodule suspension	9	2 9	3 8	3 25±0 097	0 29	1 28±0 115	0

* By litmus milk test (14)

on the agar and increase the CO_2 of the air by respiration of the glucose. Unfortunately the growth of the plants was not normal in the presence of the glucose; very short, stocky plants with a red pigment in their stalks were obtained. The nodules formed were fewer in number than on parallel cultures without glucose, but were very large. The fixation was 1.06 ± 0.33 mgm. for the plants grown with the glucose, and 2.08 ± 0.08 mgm. for parallel cultures without glucose.

The beneficial effect of plant passage on the efficiency of a root nodule bacteria has been observed in other work in which open sand pots were used to grow the plant. Also it has been noted that, in many cases, control pots that become accidentally contaminated from the air give a better fixation than those artificially inoculated. The view that the increased fixation with the

nodule suspensions is a question of strain efficiency is indicated by the erratic nature of the data; in some experiments no increase in fixation is obtained with nodule suspensions.   (See sterile nodule suspension with non-sterile seed and

TABLE 3

The effect of strain on nitrogen fixation by the clovers and alfalfa in agar

TREATMENT	NUMBER OF SAMPLES	NITROGEN PER 10 PLANTS				NITROGEN FIXED PER 10 PLANTS
		Minimum	Maximum	Average	Standard deviation	
		mgm	*mgm*	*mgm*	*mgm*	*mgm*
Experiment VI, alfalfa, 90 days						
Controls	11	1 1	1 9	1 46±0 075	0 25	
Culture 101						
Total	20	1 0	5 7	2 32±0 32	1 41	0 86±0 33
Series A	13	1 0	2 2	1 36±0 093	0 33	−0 10±0 12
Series B	7	3 0	5 7	4 10±0 355	0 94	2 64±0 34
Culture 100	19	2 6	6 4	4 50±0 255	1 11	3 04±0 27
Experiment VII, alfalfa, 75 days						
Controls	5	2 5	3 2	3 00±0 13	0 29	
Culture 101	4	3 5	6 6	5 42±0 68	1 35	2 42±0 69
Culture 100	9	4 8	7 3	6 08±0 24	0 70	3 08±0 27
Experiment VIII, sweet clover, 75 days						
Controls	5	1 9	2 8	2 24±0 17	0 38	
Culture 101	4	4 9	6 6	5 85±0 42	0 84	3 61±0 46
Culture 100	9	4 7	8 1	5 65±0 36	1 08	3 41±0 40
Experiment IX, red clover, controls and 202, 65 days, 200, 94 days						
Controls	10	1 4	2 0	1 67±0 076	0 24	
Culture 202	19	1 1	2 2	1 55±0 064	0 27	−0 12±0 10
Culture 200	17	4 9	6 9	6 08±0 146	0 58	5 41±0 17
Experiment X, red clover, 85 days						
Controls	10	1 4	2 5	1 87±0 086	0 27	
Culture 200	9	5 0	7 8	5 53±0 33	1 00	3 66±0 34
Culture 209	10	4 1	8 3	5 50±0 37	1 17	3 63±0 38
Culture 214	10	4 4	7 1	5 49±0 35	1 05	3 62±0 36
Nodule suspension	18	3 7	7 2	5 34±0 21	0 89	3 47±0 23

results of experiment X in table 3.)   In conclusion, an increased fixation of atmospheric nitrogen took place in plants grown under non-sterile conditions, but there is evidence that this result is dependent on factors that make for a more vigorous plant growth under the conditions used to exclude microorganisms, and not on the organisms *per se*.

EFFECT OF STRAIN ON NITROGEN FIXATION IN AGAR

Observations have been frequently made regarding the importance of the strain of organism with which the plant becomes infected. Frank (4) stated that legume organisms may become parasitic. Dehérain and Demoussy (3) found that white lupines possessing numerous nodules distributed over the roots contained a higher percentage of nitrogen than plants with large nodules. Helz, Baldwin, and Fred (10) reached the conclusion that large nodules, rather than smaller ones distributed over the roots were of greater benefit to a number of legumes. The literature on the subject is reviewed in the latter paper. In view of the importance of these results from a practical as well as a theoretical standpoint, a number of experiments were made in which the fixation with different strains was compared, under conditions that would preclude any possible contamination with air-borne strains of the root nodule organism. Alfalfa and sweet clover were inoculated with culture 100, a good strain; and culture 101, a poor strain. Red clover was inoculated with culture 200, a good strain; and culture 202, a poor strain. In addition, one experiment was made in which the fixation by three good strains of the clover organism and a nodule suspension were compared. The results are given in table 3. In experiment VI with alfalfa, the fixation with culture 100 was similar to that already obtained with clover. With culture 101 two types of growth resulted, viz., the plants in 13 of the 20 bottles showed signs of typical nitrogen hunger after 2 months and resembled in all respect the controls; the other seven were practically identical with the plants inoculated with the good strain, no. 100. In the table the results of the entire 20 are given as well as a subdivison into those that did (series A) and those that did not (series B) show nitrogen hunger. There was no evidence of fixation in series A, but the fixation in series B was essentially the same as that of the plants inoculated with the good strain culture 100. In further experiments (VII and VIII) made with alfalfa and sweet clover, culture 101 proved to be as efficient as culture 100. However, because of the small number of replicates in these experiments, it is doubtful whether the variation shown in the first experiment had an opportunity to exhibit itself. The erratic behavior of culture 101 suggests that with certain cultures the efficiency to fix nitrogen is a variable characteristic and fixation or lack of fixation is largely dependent on the chance distribution of the organism of the "good" fixing type. Löhnis (18) reports a similar result with strain 205 when placed on crimson clover.

With red clover (experiment IX) the usual picture of fixation with good and poor strains was obtained. Culture 200 showed a fixation of 5.41 ± 0.17 mgm. of nitrogen per 10 plants, whereas culture 202 was not significantly different from the uninoculated controls. The types of nodules obtained on the good and bad strains was similar to those described by Allen et al. (1a) except in the case of plants inoculated with culture 101. The plants which were *benefited* by this strain had both types of nodules present. The effects of inoculation with good and poor strains are shown in plate 1, figure 2.

In experiment X are given the results obtained with several good strains of the clover organisms. In work with sand cultures these often showed different efficiency of nitrogen fixation e.g., culture 209 is most efficient and is followed in order by cultures 214 and 200. In this experiment the quantities fixed by the three strains were identical. This may be due to the fact that other factors limited the growth of the plant so that the quantity of nitrogen required was such that all these good strains could supply the needs. It is apparent that growth in agar in a closed container places such limit on the development of the plant that the method is of little value in a comparison of the relative efficiency of strains except in a gross way, as with cultures 200 and 202.

NITROGEN FIXATION BY PHAGE-SENSITIVE AND PHAGE-RESISTANT STRAINS

Gerretsen et al. (5) reported the isolation of a lytic agent or bacteriophage from nodules that was specific for the bacteria of the leguminous species from which lytic agent was isolated. This finding has been confirmed by Grijns (7, 8) and Hitchner (12). The work of Hitchner (12) and Laird (16) made evident the fact that cultures of rhizobia varied in their reaction to the homologous lytic principle. Thus with a phage isolated from red clover, certain cultures isolated from the same nodule used as a source of phage were lysed by the phage while others were not. The former were termed phage-sensitive and the latter phage-resistant. Laird continued these studies and found that the different strains of the clover organism kept as stock cultures in the laboratory had different degrees of sensitivity. By plating a given culture and picking a number of colonies he was able to determine the percentage of lysis exhibited by that culture. In this way he isolated sensitive and resistant strains from a number of stock cultures. When these cultures were placed on clover plants, he noticed a difference in the type of nodule formed, a difference similar in some respects to that observed by Allen and his associates (1a) in regard to nodule distribution on plants inoculated with good and poor strains. Apparently the reaction of a culture toward the phage used was a variable characteristic of the organism since single cell cultures of a sensitive strain would give rise to resistant strains. A similar type of variability is noticed in certain cultures of rhizobia in regard to nitrogen fixation, e.g., alfalfa culture 101.

In view of these results a number of experiments were made in which the fixation by phage-sensitive and phage-resistant strains was compared. The results of these studies are given in table 4. In experiment XI a resistant strain and a sensitive strain that were separated from a culture isolated from the nodule suspension used for the phage isolation were compared. The results showed that neither strain was able to fix nitrogen on the plant although abundant nodules were formed. Other experiments in which the phage was added at the time of inoculation confirmed this result. It was later found that the culture from which these strains were isolated was a poor type similar to

alfalfa culture 101, e.g., with some plants fixation occurred, whereas in others little or none took place. Another experiment (XII) was made in which phage-sensitive and phage-resistant strains isolated from stock cultures that

TABLE 4

Nitrogen fixation by phage-sensitive and phage-resistant strains of red clover

TREATMENT	NUMBER OF SAMPLES	NITROGEN FIXED PER 10 PLANTS				NITROGEN FIXED PER 10 PLANTS
		Minimum	Maximum	Average	Standard deviation	
		mgm	*mgm*	*mgm*	*mgm*	*mgm*
Experiment XI, 72 days						
Controls	5	1 4	1 9	1 70±0 084	0 19	
Culture 29						
Resistant	5	1 5	1 9	1 66±0 046	0 14	−0 04±0 095
Sensitive	5	1 6	1 9	1 76±0 064	0 10	+0 06±0 106
Experiment XII, 71 days						
Controls	5	1 9	2 8	2 14±0 17	0 38	
Culture 205	7	5 4	9 2	7 06±0 50	1 33	4 92±0 53
R 205-3*	6	7 1	9 5	7 38±0 58	1 41	5 24±0 60
S 205-21†	5	4 6	7 1	6 32±0 30	0 68	4 18±0 34
Mixture R and S	7	5 9	8 7	6 66±0 45	1 10	4 52±0 48
Culture 209	6	4 5	7 7	6 13±0 53	1 30	3 99±0 56
R 209-12*	6	3 4	9 0	5 92±0 84	2 06	3 78±0 86
S 209-4†	5	6 1	8 0	6 58±0 36	0 81	4 44±0 40
Mixture R and S	7	4 5	6 8	5 57±0 33	0 86	3 43±0 37
Culture 214	6	5 6	7 5	6 43±0 29	0 72	4 29±0 34
R 214-4*.	6	5 8	9 2	6 75±0 57	1 39	4 61±0 59
S 214-22†	5	5 6	8 2	6 60±0 46	1 03	4 46±0 49
Mixture R and S	6	4 6	9 6	6 60±0 75	1 85	4 46±0 77
Culture 218	5	5 6	7 6	6 06±0 44	0 99	3 92±0 47
R 215-9*	5	4 9	6 8	5 78±0 32	0 71	3 64±0 36
S. 215-18 † No nodules formed						
Mixture R and S	5	4 7	6 4	5 20±0 32	0 71	3 06±0 36

* Resistant.
† Sensitive.

were known to be efficient nitrogen-fixing strains were used. In this experiment there were included plants inoculated with the original culture as well as mixtures of the phage-resistant and phage-sensitive strains. The reaction toward the phage of the organisms used is:

Culture 205.   Good nitrogen fixer.   40 per cent sensitive
               Substrain 205–3.   No clearing (resistant)
               Substrain 205–21.   Partial clearing (partially sensitive)
Culture 209   Best nitrogen fixer.   36 per cent sensitive
               Substrain 209–4.   Partial clearing (partially sensitive)
               Substrain 209–12.   No clearing (resistant)
Culture 214.   Good nitrogen fixer.   68 per cent sensitive
               Substrain 214–4.   No clearing (resistant)
               Substrain 214–22.   Complete clearing (sensitive)
Culture 215.   Good nitrogen fixer.   92 per cent sensitive.
               Substrain 215–9.   No clearing (resistant)
               Substrain 215–18.   Complete clearing (sensitive)

The reactions of the original culture toward the phage are taken from Laird's data[3] and were obtained by picking 25 colonies from a plating of each culture and testing these substrains against the phage; if a substrain showed complete or partial clearing during a 120-hour period, it was counted as sensitive. From some of the cultures it was possible to obtain substrains that were completely lysed by the phage whereas from others all substrains were either completely resistant or partially so.

The results of experiment XII (table 4) show that the quantity of nitrogen fixed by the sensitive and resistant strains did not differ significantly from that of the original stock culture. Culture 215–18 did not produce nodules although the organisms were obtained from the agar at the end of the experiment. This occurrence of a non-nodule producing strain has been observed on several occasions and is now under investigation in this department. In order to put all of the cultures under comparable greenhouse conditions, it was necessary to limit each set to about six bottles and thus the variability of the results was somewhat high. However, it is felt that sufficient samples were taken to establish that apparently reaction to this particular race of phage is not correlated with efficiency as nitrogen fixers. However, it is not claimed that these data completely settle this point. In all of the tests only one phage was available and it is possible that the cultures would give an entirely different reaction if other races of phage were used. Also the great variability of a given culture (even when picked from a single cell) toward the phage raises the question as to type of organism that *invades* the plant [Laird (16)]. It appears that both sensitive and resistant strains can arise from any culture so that the use of a "sensitive" or a "resistant" culture (which apparently means only predominating strains) for inoculation does not *insure* invasion of the plant by the type that is present in the largest number. This work is being continued and further reports will be made as it progresses.

SUMMARY

Nitrogen can be fixed by clover and alfalfa root-nodule bacteria in conjunction with the proper host plant in an agar substrate and under conditions

that exclude the presence of any other organisms. The presence of ordinary air contaminators does not appear to affect the fixation in any way.

The quantity of nitrogen fixed in an agar substrate in bottles closed with cotton plugs varied from 2 to over 10 mgm. per 10 plants. The quantity of nitrogen fixed appears to be dependent on the general growth of the plant. Under the experimental conditions employed the plant growth is limited more by factors such as light, gas exchange, and transpiration than by a need for nitrogen, hence the latter is the dependent variable rather than the independent one.

There was evident an increase in the nitrogen fixed by plants from unsterilized seeds or inoculated with unsterilized nodule cultures. However, this increase may be due to effects tending to give increased plant growth rather than to the presence of other organisms as such.

The effect of inoculation with good and poor strains was demonstrated under conditions that excluded any chance contamination. The use of the agar culture technique for differentiating efficiencies of a number of strains is apparently limited to extremes of "good" and "poor," since factors other than need for nitrogen influence the growth of the plants to an extent that small differences in nitrogen-fixing ability are masked.

Preliminary studies on the relationship between the behavior of a culture toward a phage and its nitrogen-fixing ability indicate no correlation, but further studies of this question are necessary.

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PLATE 1

FIXATION OF NITROGEN BY CLOVER IN AGAR SUBSTRATE

FIG. 1. The influence of inoculation on clover in agar substrate.

FIG. 2. The fixation of nitrogen by effective and ineffective clover strains in agar substrate.

P W WILSON, E W HOPKINS AND E. B FRED

Control   Culture 200

Fig 1
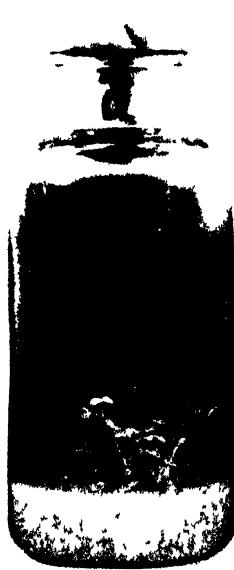
Clover culture 200   Uninoculated control   Clover culture 202
Effective strain   Ineffective strain

Fig 2

CHANGES IN COMPOSITION OF SOYBEANS TOWARD MATURITY AS RELATED TO THEIR USE AS GREEN MANURE

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The composition of a plant is a significant factor in determining its usefulness as a green manure. Legumes make excellent green manure, ostensibly on account of their high content of nitrogen, whereas straw is detrimental on account of its low analysis with respect to this element. Soybeans are often a satisfactory green manure, for as a legume they are rich in nitrogen; yet it has been reported that the wheat crop following soybeans often gives lower yields than when not preceded by this crop. Thatcher (2) of Ohio, reports that the yield of wheat following soybeans cut for hay decreased as the date of the harvest of the soybeans was deferred. There was also a decrease in the percentage of nitrogen in the soybean roots with advancing maturity. The value of a legume for green manure depends not only on the mere presence of a certain amount of nitrogen, but also on the release of that nitrogen in a soluble form after the crop is turned under. The composition of the green manure in other respects than nitrogen, e.g. its carbon as related to nitrogen, is important. In fact, its composition as combined with that of the soil to make this a fit microbiological medium for its decomposition and nitrogen release is the final index of its value as a green manure. Since the speed and nature of the decomposition of the plant vary with maturity, according to Waksman (5), it is essential to learn more about the composition of the soybean as a possible explanation for the irregularities in its effects on succeeding crops.

The following study is an attempt to measure by chemical means the differences in organic composition of the tops and roots of the soybean plant, which accompany increasing maturity, in the belief that they may offer suggestions regarding the decomposition behavior of these plant parts in the soil.

PLAN OF STUDY

The ease of digestion by chemical reagents was taken as a rough criterion of the readiness with which microbiological digestion, or decomposition, will take

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place within the soil.   The study was based on the relative degree of solubility of the soybean plant material when the intensity of the successive chemical treatments was increased.   The decomposition rate as related to chemical condition of such plant material, may be roughly divided into three divisions, as follows:

Rapidly decomposable material—that which is soluble in water.
Slowly decomposable material—that which is insoluble in water, but soluble in dilute alkali and acid.
Non-decomposable material—the residue of the aforementioned treatments, or that which is soluble in ether and strong acids.

Analytical determinations were made on the soybean materials after they had been grouped according to these treatments, or as they arranged themselves according to the ease of chemical attack.   Special attention was given to the nitrogen and carbohydrates present in each group, since they play such an important part in microbial activity.

PREPARATION OF MATERIALS

The soybean plants used were collected during 1929 from fertile, well-inoculated Shelby loam soil that had been seeded with the Morse variety of soybeans on June 6.   The beans were kept well cultivated throughout the summer.   Beginning July 6, when the plants were about 15 inches high, a portion was harvested and at regular intervals thereafter until October.   The complete plant was taken, and divided into roots and tops by cutting it about 3 inches above the ground to approximate field cutting by machine.   After air-drying, these portions were ground and preserved for later study.

The summer was rather dry.   The lack of rain of any significance during August caused the dropping of many leaves.   During the first week in September a rainfall of 2 inches stimulated the vegetative growth.   The moisture content during the season of a soil receiving similar tillage is given in figure 1.

LABORATORY METHODS

All determinations were made on the oven-dry basis, by drying the material to constant weight at 105°C. and determining the percentage of moisture.   All total nitrogen determinations were made by the Kjeldahl method.   The chemical treatments given the plant materials for partial digestions were those outlined by Waksman (4).   The sample was first treated with ether to remove the fats and waxes which decompose very slowly in the soil and may cover other plant constituents so that the microörganisms cannot attack them. The residue from the ether extraction was treated with cold water, removing the easily soluble and readily decomposable material.   This group includes the simple carbohydrates, their derivatives, various amino acids, water-soluble nitrogen compounds, and soluble minerals.   The residue from the cold water extraction was then extracted with hot water, removing some of the less read-

ily decomposable materials including the starches, some pectins, certain hexoses, and various nitrogen compounds.

In order to determine the slowly decomposable group of substances, the plant residue from the hot water extraction was treated with a 5 per cent solution of sodium hydroxide at high temperature under pressure. This extraction contained alkali-soluble lignin [constituting, according to Waksman (4), about 20 per cent of the total lignin], certain hemicelluloses, and the more indifferent nitrogen compounds. The residue remaining after this treatment by the sodium hydroxide was then treated with 2 per cent sulfuric acid at
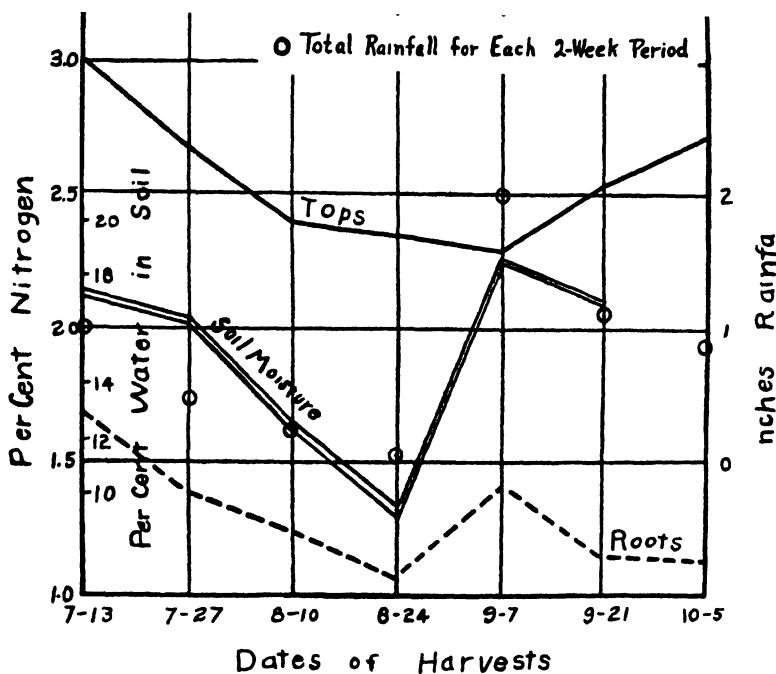
FIG. 1. NITROGEN CONTENT OF SOYBEANS DURING THE SEASON AS CORRELATED WITH SOIL MOISTURE

boiling temperature, removing another portion of hemicellulose of slow decomposition rate.

The residue from the sulfuric acid extraction was treated with ammoniacal copper sulfate (modified Schweitzer's reagent) for the determination of cellulose, whose microbiological breakdown in the soil is highly dependent on the supply of readily available nitrogen (3). The pentosan portion was determined by transforming it into furfural on heating with acid. This serves as a source of energy for soil microörganisms, and its ratio to lignin is, according to Rege (1), the basis for the rate of decomposition.

The undecomposable material, or lignin, was determined by freeing an un-

treated sample of material of its fats and waxes, and then treating it with a mixture of 18 per cent hydrochloric acid and 72 per cent sulfuric acid combined in the proportion of 1:5 respectively. This residue was classed as lignin, which decomposes very slowly, or probably not at all in the soil.

These determinations were made on soybean plant parts harvested every two weeks from July to October, as a means of determining the changes in organic composition with advancing maturity, as such might indicate variation in the amounts of decomposable and non-decomposable substance.

ANALYTICAL RESULTS

The total nitrogen in the tops fluctuated with the seasonal conditions, decreasing with the dry weather and dropping of leaves in August, and increasing again with the Autumn rains stimulating growth. In the roots there was a continual decrease until late August and then a sudden rise in nitrogen content, followed by a decrease toward maturity. These data are assembled in table 1 and correlated with the moisture in figure 1.

TABLE 1

Total nitrogen in the tops and roots of soybeans at varying stages of growth

(Per cent of water-free sample)

DATE OF HARVEST	TOTAL NITROGEN		DATE OF HARVEST	TOTAL NITROGEN	
	Tops	Roots		Tops	Roots
July 13 .	3 015	1 690	September 7	2.299	1 397
July 27 . . .	2 662	1 378	September 21	2.547	1.156
August 10 .	2.406	1 239	October 5.....	2.728	1 147
August 24	2.375	1.067			

The data of the analytical results for the digestive treatments, as expressed in terms of percentage of sample, are assembled in table 2 for the tops and roots of the soybean plants. The ether-soluble content, or fats and waxes, in the roots decreases gradually with maturity. In the tops there is also a decrease, with the lowest amount in late August and early September, possibly in consequence of the low rainfall of August.

For both the cold and hot water fractions there are reported four subdivisions; namely, the total substance soluble, the ash, the nitrogen, and the reducing sugars. The reducing sugar dissolved by hot water is that which was hydrolyzable by boiling in 2 per cent hydrochloric acid. According to the data for the cold water extraction, the solubility of both the tops and roots decreases with maturity. There is a corresponding decrease for both the ash and the nitrogen. There is, however, a decided increase with maturity for the cold water-soluble reducing sugar. This decrease in soluble nitrogen and the increase in soluble sugar, or carbohydrates, are significant with reference to changes in the nature of decomposition as the plant becomes more mature. It

TABLE 2

Composition of soybeans according to chemical digestion, as related to maturity of plants

(Expressed as per cent of total water-free initial sample)

FRACTION		JULY 13		JULY 27		AUGUST 10		AUGUST 24		SEPTEMBER 7		SEPTEMBER 21		OCTOBER 5	
		Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots
Ether-soluble	Total substance	5 42	3 40	5 02	3 15	4 96	2 98	2 32	2 43	1 73	1 12	3 85	1 47	3 18	0 22
Cold water soluble	Total substance	19 72	12 37	18 55	10 91	16 99	10 63	15 24	10 63	17 07	10 66	19 98	11 85	18 25	11 68
	Ash	6 18	4 34	5 20	2 41	4 82	3 68	6 07	2 91	5 33	2 96	5 54	2 90	4 59	1 79
	Nitrogen	0 92	0 54	0 90	0 31	0 74	0 35	0 91	0 32	0 83	0 32	0 64	0 32	0 52	0 31
	Reducing sugar	7 93	3 35	4 91	3 82	8 51	3 64	6 94	4 53	10 66	8 57	11 38	8 71	12 49	8 62
Hot water-soluble	Total substance	5 14	2 41	6 94	3 72	5 88	3 47	2 72	1 75	2 55	2 78	3 13	3 03	4 79	3 67
	Ash	1 09	0 39	1 70	0 79	1 33	1 11	0 80	0 53	0 68	0 64	0 81	0 47	0 76	0 45
	Nitrogen	0 24	0 14	0 26	0.13	0 26	0 06	0 16	0 13	0 14	0 10	0 15	0 13	0 18	0 15
	Reducing sugar	3 52	1 57	3 68	2 87	2 37	2 54	2 51	1 35	2 24	1 95	2 09	2 19	2.13	2 54
Alkali-soluble	Total substance	33 58	27 18	32 76	24 69	33 70	28 36	35 49	30 92	36 68	32 09	36 61	31 80	44 70	33 40
	Nitrogen	1 10	0 60	0 95	0 51	1 02	0 56	0 94	0 52	1 00	0 65	1 21	0 58	1 12	0 45
	Lignin (protein free)	2 53	1 90	2 78	1 97	5 11	2 36	2 84	1 83	2 70	1 72	2 97	2 67	2 16	2 06
	Reducing sugar	21 37	12 26	22 63	14 36	20 09	15 84	16 75	17 06	16 75	23 91	18 87	26 38	21 47	29 30
Acid-soluble	Total substance	2 47	2 94	2 64	2 99	3 13	3 01	3 19	3 52	3 59	4 41	4 43	4 37	4 67	4 63
	Reducing sugar	0 49	0 58	0 52	0 59	0 62	0 60	0 64	0 70	0 72	0 88	0 88	0 91	0 93	0 92
Cellulose		10 67	20 02	13 69	19 89	13 69	19 73	13 99	19 56	14 28	20 83	14 49	21 74	14 99	22 49
Pentosans		9 74	12 96			10 66	15 25			10 99	14 46			11 94	14 96
Lignin (ash and protein free)		11 88	9 12	12 91	18 32	13 76	17 39	15 79	17 41	18 91	17 38	22 51	17 77	23 03	22 99

designates a widening nitrogen-carbon ratio, indicating that there would be a smaller amount of soluble nitrogen liberated from the decay of material of the more mature harvest. This decrease in available nitrogen and increase in carbohydrates with maturity, especially in the roots with their already wide nitrogen-carbon ratio, may leave such excessive amounts or readily decomposable carbohydrates and such deficient amounts of available nitrogen, that the decomposition of the carbohydrates removes the soluble nitrogen from the soil and injures the succeeding crop.

The data for the hot water extract bear some resemblance to those for the total substance, the ash, and the nitrogen of the cold water treatment, but the changes with maturity are by no means so extreme. The hot water-soluble nitrogen in the roots is very low and fluctuates little through the season. The reducing sugar extracted from the roots increases but slightly, whereas that from the tops decreases slightly with maturity—the opposite of that for cold water extraction.

The fraction removed by sodium hydroxide and containing the alkali-soluble lignin, which makes up less than 20 per cent of the total lignin (5); certain hemicelluloses; and the more complex nitrogenous compounds, increases with maturity for both tops and roots. This is the reverse of the amounts soluble in cold water. The percentage of nitrogen soluble in alkali is almost constant, which may also be said of the lignin content of this fraction. The reducing sugar content of the tops remains low through the season, whereas that in the roots increases decidedly. Here again, in the roots in particular, the nitrogen remains constant while the carbohydrate increases with maturity, agreeing with the results of Waksman (5) for rye plants. This widening of the nitrogen-carbon ratio lessens the readiness of nitrogen liberation when this difficulty soluble material is decomposed in the soil.

A relatively small amount, only 2.0 to 5.0 per cent of the residue after alkali treatment, was soluble in sulfuric acid. According to the data, the total soluble substance and the reducing sugar increase with maturity in case of both tops and roots. Again in this fraction, the carbohydrate increases as the plant becomes older.

The study of the cellulose, determined by the method of Waksman (4), shows that this increases in the tops with maturity, going from over 10 per cent to 15 per cent. The roots' content of this compound remains nearly constant at the much larger figure of 20 per cent, reaching the maximum of 22.5 per cent on the last harvest date of October 5. Since the decomposition of cellulose demands the presence of certain minerals, but especially of available nitrogen, it is interesting to note the ratio of cellulose to total nitrogen in these samples at these various dates through the growth period as shown in figure 2. In the tops, this ratio was near 5:1 at the beginning, showing an increase and then a decrease with time. The roots, however, show a much wider ratio of 11:1 at the outset, becoming as wide as 19:1 in the late harvest. In comparison with the ratio of 50:1 cited by Waksman as the ratio of cellulose to available nitro-

gen facilitating cellulose decomposition, this suggests that if the supply of total nitrogen in the soybean material is all readily available it is ample for the decomposition of the cellulose. Since a good portion of the total nitrogen is combined into very stable compounds, the ease of decomposition cannot be predicted by this ratio.

It was suggested by Rege (1) that two factors control the biological destruction of cellulose, namely, energy material as pentosans and an "inhibitory factor" as lignin. The pentosans of the soybean plants were determined by the usual phloroglucide method on the harvests of 4-week intervals. This constituent increased decidedly with maturity. The lignin determination showed that this organic substance almost doubled itself, going from about 12 to more than 23 per cent in the tops, whereas in the roots it was 9 per cent at the first
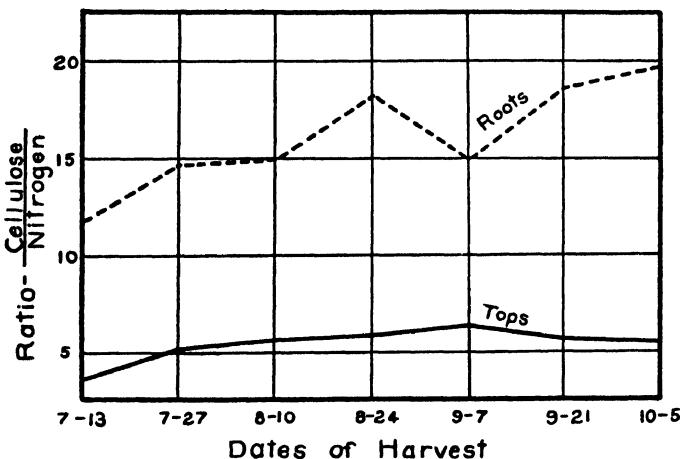
FIG. 2. CELLULOSE-NITROGEN RATIO OF SOYBEANS DURING THE SEASON

harvest, and increased to almost 18 for the rest of the dates except the last one, when it mounted to 23 per cent. Since lignin is not digested by 72 per cent sulfuric acid and is considered decomposable in the soil only very slowly, the more mature soybean plant plowed into the soil adds about one-fourth of its organic matter in a slowly decomposable form.

Rege (1) believes that cellulose decomposition depends on the pentosan-lignin ratio. This, in his opinion, should be greater than 1.0:1.0 for rapid decomposition whereas a ratio falling below this as far as 0.5:1.0 signifies slow decomposition. These ratios for the soybean plant roots at all times and for the tops on all later dates fall below the figures 1.0:1.0 and would—if Rege's deductions are significant—indicate slow decomposition of their cellulose fraction in the absence of other sources of energy.

Since the nutrient elements within the lignin are poorly available on account of its lack of decomposition, a determination of the lignin nitrogen was

made to learn how much of this element is locked up in the inert form. This remained fairly constant throughout the growth period, as shown in table 3. According to Waksman and Tenney (5), lignin contains 63 per cent carbon. Using this figure as a basis for calculation, it was found that this undecomposable or lignin carbon increases from 7.5 in the young plant to 14.5 per cent at maturity. With the content of nitrogen in the lignin remaining constant while the carbon increases, the nitrogen-carbon ratio of the lignin becomes correspondingly wider.

Because the carbon and nitrogen within the lignin are so inert, it was deemed well to subtract the percents of these two elements from their total amounts

TABLE 3

Composition of lignin of soybeans with increasing maturity

(Expressed as per cent based on lingin sample)

		JULY 13	JULY 27	AUGUST 10	AUGUST 24	SEPTEMBER 7	SEPTEMBER 21	OCTOBER 5
Ash	Tops	3 33	0 62	5 01	1 49	0 64	1 52	1 19
	Roots	6 71	3 67	4 39	4 53	4 87	8 32	10 63
Carbon*	Tops	7 48	.	8 67		11 91	.	14 50
	Roots	5 74		10 95	.	10 95		14 48
Nitrogen	Tops	0 49	0 61	0 41	0 45	0 44	0 49	0 48
	Roots	0 32	0 42	0 40	0 28	0 29	0 44	0 38
Protein†	Tops	3 10	3 80	2 60	2 82	2 75	3 09	2 99
	Roots	2 01	2 66	2 54	1 77	1 85	2 76	2 36
Carbon‡	Tops .	39 40	40 92§	42 43	42 99§	43 54	44 59§	45 64
	Roots	40 16	41 65§	43 14	43 42§	43 71	44 15§	44 58

* Lignin × 0 63.

† Nitrogen × 6 25.

‡ Based on original water-free sample of plant material (data by L. M. Turk).

§ By interpolation from preceding and succeeding dates.

(total nitrogen, table 1, total carbon table 3) and to designate the resultant values as "decomposable carbon and nitrogen." The relationship of the carbon to the nitrogen as they are considered decomposable on this basis is expressed by the results in figure 3. These ratios are almost a duplicate of those for the total carbon to the total ntrogen in figure 4. If such conditions prevail in all plants and the assumed "decomposable carbon and nitrogen" are in accord with the facts, one might obtain an index of decomposability by simple determinations of carbon in green manures. It is significant that these ratios for the tops became widest for the early week in September, associated with the extreme of the drought, and then became narrower again with the

fall rains. In the case of the roots the reverse fluctuation occurs. Starting at a ratio of 25:1, it becomes wider then narrower and finally widens to its maximum of 39:1 in the last harvest. Since the narrower carbon-nitrogen ratio speeds decomposition (3), these soybean roots with their wide ratio would
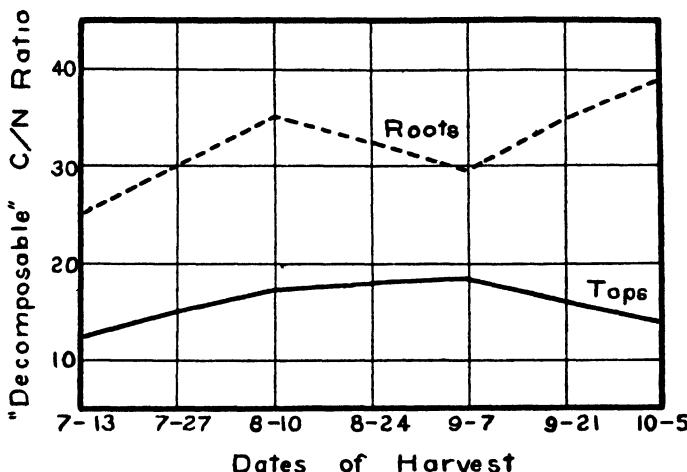
FIG. 3. RATIOS OF CARBON TO NITROGEN AS CONSIDERED "DECOMPOSABLE" FOR SOYBEANS
DURING THE GROWTH SEASON
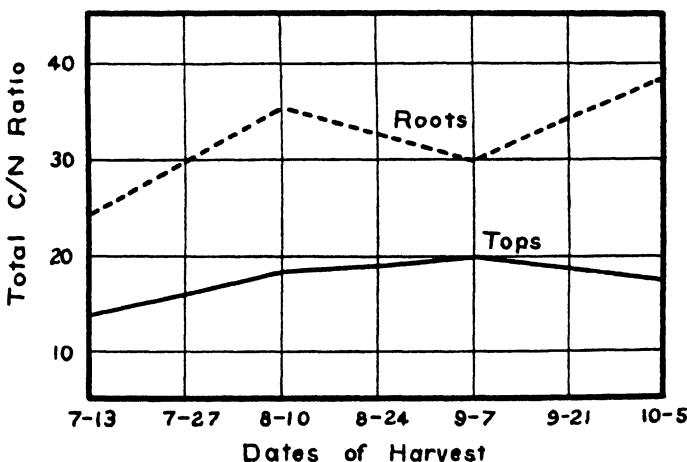
FIG. 4. RATIO OF CARBON TO NITROGEN OF SOYBEANS DURING THE GROWTH SEASON

demand an additional source of available nitrogen for rapid and complete decomposition. This nitrogen must necessarily come from the soil and, in consequence, the crop following soybeans may suffer because the decaying soybeans roots are competing with it for the soil's supply of available nitrogen.

Since microbiological digestion depends mainly on energy material, which may be found in the reducing sugars, the cellulose, and the pentosans; on the growth material, or nitrogen, which for purpose of calculation we shall consider as supplied mainly by the water-soluble and alkali-soluble nitrogen (6) in these data; and on some ash, assumed plentifully present, let us note the changes in amounts of energy material and nitrogen so supplied by the tops

TABLE 4

Readily soluble nitrogen and energy materials of soybeans as related to maturity of plants

(Expressed as per cent of total water-free sample)

		JULY 13	JULY 27	AUGUST 10	AUGUST 24	SEPTEM BER 7	SEPTEM BER 21	OCTO BER 5
		Tops						
Nitrogen	Cold-water soluble	0 92	0 90	0 74	0 91	0 83	0 64	0 52
	Hot-water soluble	0 24	0 27	0 27	0 16	0 14	0 15	0 18
	Alkali-soluble	1 11	0 95	1 02	0 94	1 01	1 22	1 13
	Total readily soluble	2 27	2 12	2 03	2 01	1 98	2 01	1 83
	Balance—inert	0 74	0 54	0 38	0 35	0 32	0 54	0 90
Reducing sugars		33 33	31 76	31 61	26 85	30 39	33 24	37 04
Cellulose		10 67	13 69	13 69	13 99	14 28	14 49	14 99
Pentosans		9 74	10 20*	10 66	10 83*	10 99	11 47*	11 94
Total energy materials		53 74	55 65	55 96	51 67	55 65	59 20	63 98
Ratio $\dfrac{\text{Energy}}{\text{Soluble nitrogen}}$		23 6	26 2	27 5	25 6	28 1	29 4	34 8
		Roots						
Nitrogen	Cold-water soluble	0 54	0 31	0 36	0 32	0 32	0 32	0 32
	Hot-water soluble	0 15	0 13	0 07	0 13	0 10	0 13	0 15
	Alkali-soluble	0 60	0 52	0 56	0 52	0 66	0 59	0 45
	Total readily soluble	1 29	0 96	0 99	9 97	1 08	1 04	0 92
	Balance—inert	0 41	0 42	0 25	0 10	0 32	0 11	0 13
Reducing sugars		17 77	21 67	22 64	23 66	35 32	38 20	41 40
Cellulose		20 02	19 89	19 73	19 56	20 83	21 74	22 49
Pentosans		12 97	13 11*	13 25	13 86*	14 47	14 71*	14 96
Total energy materials		50 76	54 65	55 62	57 08	70 62	74 65	78 85
Ratio $\dfrac{\text{Energy}}{\text{Soluble nitrogen}}$		39 6	56 5	55 7	58 8	65 3	71 5	85 7

* Interpolated from preceding and succeeding dates.

and roots as the plant changes with maturity. The sum of the sugars, pentosans, and cellulose is taken as energy material even though these may each have been partly included in other than its own determinations. The data assembled for the aforementioned purpose are given in table 4. The outstanding fact in these calculations is the change in the ratio of energy material to the nitrogen in the tops, but especially in the roots. In the tops this increases

from 23.6 to 34.8 during the season, whereas in the roots these figures go from 39.6 to 85.7. For the decomposition of cellulose alone, Waksman (3) cites the need of one part of available nitrogen for each 50 to 60 parts of cellulose. In the more mature soybean roots the ratio is wider than this when even partially stable nitrogen is included. Certainly this wide ratio suggests that the soybean roots supply far too great an amount of energy material in proportion to their nitrogen or growth material for ready decomposition, and suggest that such mature roots turned under would draw on the soil's supply of soluble nitrogen and endanger the succeeding crop.

SUMMARY AND CONCLUSIONS

A chemical study of the organic constituents of the tops and roots of soybeans with advancing maturity of the plants suggests that the effectiveness of this crop as a green manure to liberate nitrogen in the soil will vary widely with different stages of growth. The following facts are of interest:

The percentage of total nitrogen decreased with the age of the plant. In the roots it was only in the earliest part of the season that it reached the figure 1.70, considered by Waksman (5) as the minimum at which decomposition with nitrogen liberation occurs.

The water-soluble constituents decreased in both roots and tops as the plant became older. Less than 30 per cent of the total nitrogen was water soluble, or in the form considered by some as readily nitrifiable.

The alkali-soluble material, considered slowly decomposable, increased markedly toward plant maturity and the nitrogen content of this portion decreased, especially in the alkali-soluble lignin.

The carbonaceous material, including the reducing sugars, the cellulose, and the pentosans, all decomposable by soil microorganisms in the presence of soluble nitrogen, increased with maturity of the plant.

In consequence of the increase of carbonaceous matter and the decrease in total nitrogen, there was a widening carbon-nitrogen ratio. The study shows a far more rapidly widening ratio when the carbonaceous matter and that nitrogen which is more readily soluble are used in its calculation.

The percentage of total lignin, decomposable only slowly, increased over 100 per cent in 3 months of growth. In the tops, this increased from less than 12 to almost 24 per cent, and in the roots it mounted from about 9 to almost 23 per cent during this time. The pentosan-lignin ratio was a narrow one, which might indicate slow decomposition, especially of the soybean roots.

The depressing effect of soybeans on the crop which follows is readily possible in consequence of the large percentage of carbonaceous matter and the small percentage of readily usable nitrogen which the mature soybean crop—especially the roots—supply to the soil microörganisms. This study indicates that the percentage of more readily soluble nitrogen in the soybean crop, including both tops and roots but especially roots, decreases as the plants grow older, whereas that of the carbonaceous matter usable by microörganisms mounts so rapidly that, as a green manure, the more mature crop is a bacterial ration with excess of the carbonaceous matter and such deficiency of readily soluble nitrogen that its decomposition may jeopardize the soil's supply of

available nitrogen for other crops.   There is need for fuller chemical knowledge of the changes of the organic complexes of the plant as it becomes more mature, and for a fuller knowledge of the decay of these complexes within the soil, so that chemical studies of green manures may more wisely guide their use for maximum effective nitrogen liberation in the soil.

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THE EFFECTS OF VEGETATION AND CLIMATE UPON SOIL PRO-FILES IN NORTHERN AND NORTHWESTERN WYOMING

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During the summer seasons of 1927, 1928, and 1929 soil surveys were made of the agricultural lands of the Big Horn Basin and Sheridan County in Northern and Northwestern Wyoming. Practically all of the mature soils of the Big Horn Basin are fundamentally the same from a scientific standpoint, but as one ascends the surrounding foothills and mountains, striking differences in soils and in vegetation may readily be observed.[1]

Many botanists and zoologists have recognized and described the variations of plant and animal life which are encountered as one ascends the slopes of high mountains to progressively cooler and moister regions, and many scientists have recognized that soils vary, in their fundamental characteristics, in a similar way. Glinka (4) and other eminent European soil scientists have recognized the existence of soil zones paralleling climatic zones in the mountains and have written more or less voluminously on the subject. In the United States but little detailed study has been made of this phenomenon although a number of scientists have recognized its existence. Because of the limited time at the disposal of the writer the studies are rather limited in scope, but sufficient data were gathered to be used in forming a fairly comprehensive idea of the processes which have been, and are at present, contributing to the nature of the mature soils of this region.

The territory under discussion includes: first, the terraced lands of the Big Horn Basin from Worland and Tensleep north to Lovell and northwestward to Powell, Elk Basin, and Cody; second, the western slopes of the Big Horn Mountains from Hyattville to Shell, which border Big Horn Basin on the east; third, Yellowstone Park, which is separated from the Big Horn Basin by the Absaroka Mountains (a range of the Rocky Mountain system); and fourth, the eastern slope of the Big Horn Mountains and the edge of the great plains which surround the cities of Buffalo and Sheridan.

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[1] While working on the soil surveys the writer spent many week-ends in taking trips into the mountains to gather field notes and soil samples to show these differences. He was accompanied and assisted on some of these trips by Mr. E. G. Fitzpatrick of the Bureau of Chemistry and Soils and on two or three occasions by Mr. T. J. Dunnewald of the Wyoming Agricultural Experiment Station.
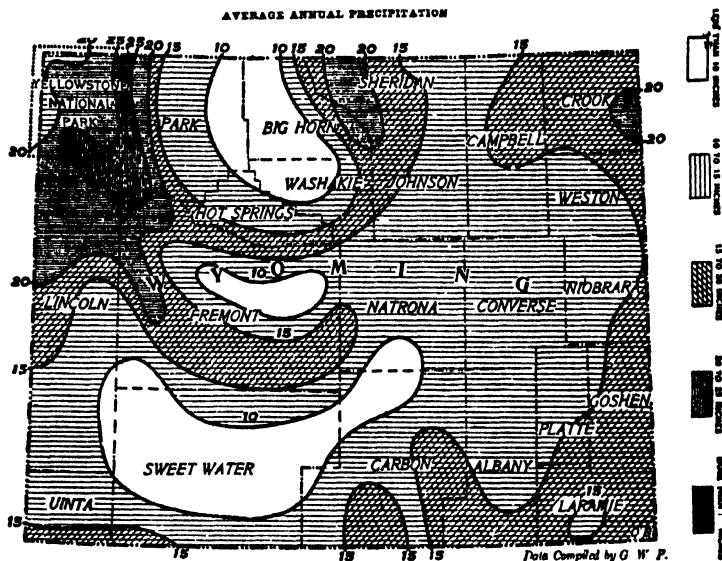
FIG. 1
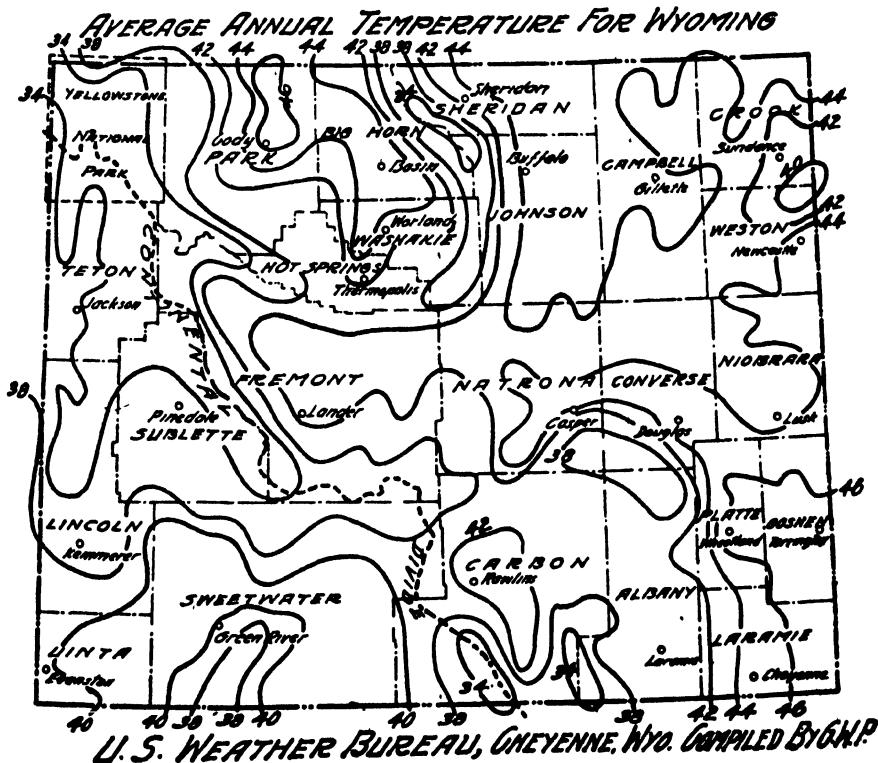
FIG. 2

CLIMATIC VARIATIONS

The variations in precipitation in the part of Wyoming which we are considering may be illustrated most briefly by the accompanying Weather Bureau map of the State (fig. 1) with a few statistics from the same source (9). A study of Weather Bureau figures (9) shows an average precipitation of approximately 6 inches for the larger part of Big Horn Basin, which places it in the

Average Annual Precipitation in Yellowstone National Park
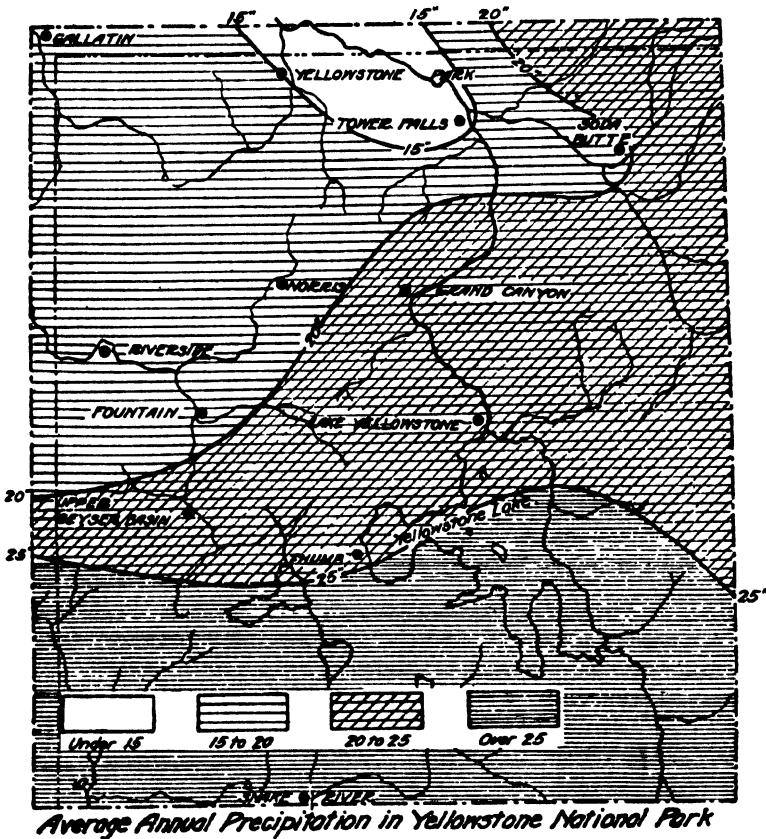
class of true deserts. Surrounding Big Horn Basin are concentric bands of territory which have progressively greater amounts of precipitation until the higher peaks of the Absarokas on the west, and of the Big Horns on the east are reached. The maximum annual precipitation of which there is record in the Big Horns is 24.74 inches at Dome Lake (9). Since this station is centrally located it probably closely approximates the average rainfall for the major part of the mountains. Estimates of as high as 40 inches per annum have

been made by various scientists but it is doubtful whether there is that amount of rainfall in any considerable area in the Big Horns. A precipitation map of Yellowstone Park (3) is presented (fig. 3) to supplement the Wyoming map. From this it may be seen that the average annual rainfall considerably exceeds 25 inches in the Absarokas. From the mountain tops the total rainfall decreases westward and eastward respectively with the corresponding decreases in altitude. A study of the isothermal lines (9) showing average annual temperatures for Wyoming (fig. 2) shows that they are distributed in positions roughly paralleling the moisture belts, with the cooler portions lying at the higher altitudes. With this in mind one is warranted in presuming that moisture conditions in the soil will vary even more than one would expect from the mere difference in rainfall, that is to say, since the warmer temperatures prevail where the precipitation is least, the evaporation will be greater at this point also, and the soil will retain an even smaller proportion of the water it receives than will the soil in the more humid regions of the mountains. The regions of lower altitude in Big Horn Basin, and in the vicinity of Sheridan, have cold winters, cool autumns and springs, and short warm summers. The temperature variations in the mountains are less pronounced. The winters are very cold and the snow banks remain until late in June on the higher slopes. A few of the peaks have perpetual snows on their more sheltered slopes. Snowstorms are likely to occur any month of the year and there are usually fairly heavy snowfalls as early as the latter part of September.

SOILS AND VEGETATION

It is not the object of this paper to go into great detail regarding the minute differences of structure and color which were observed in the great mass of profiles examined. These may be studied in part at least in the soil survey reports of the Shoshone, Basin, and Sheridan County areas which will be published at a later date. It is our purpose rather, to describe, in brief, typical soil samples taken from the different soil belts and endeavor to show their relations to climate and vegetation. The writer is also endeavoring to correlate his findings with the classification of the great soil groups of the world as outlined by Glinka (4) and by Marbut (5). We shall begin with the desert soils of the Big Horn Basin and trace the changes eastward and westward from this locality.

Mature soils in the Big Horn Basin are found lying chiefly on a series of gravel terraces which were laid down in Quarternary times (7). These terraces range from 20 to several hundred feet above the levels of the chief river courses of the Basin and constitute a large portion of the higher lands. There are also fairly large areas of rolling uplands which are underlaid by sandstones and shales. Mature soils are often found on the sandstones but the shales are so resistant to weathering that soils derived from them are usually very thin and immature.

The vegetation on the desert is so sparse that it is easy to observe the general
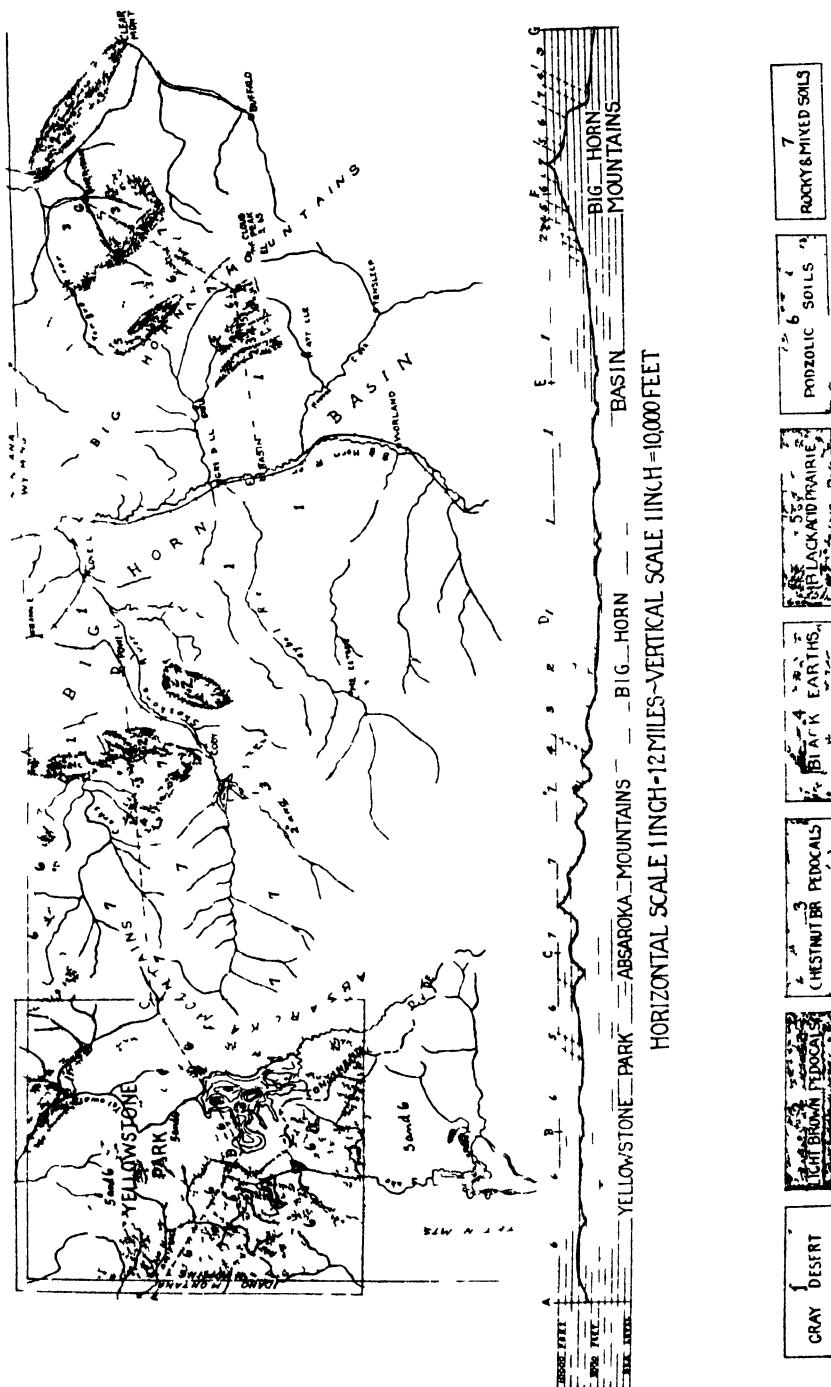
FIG 4 MAP AND CROSS SECTION OF NORTHWESTERN AND NORTH CENTRAL WYOMING SHOWING SOIL BELTS AND THEIR RELATION TO ALTITUDE

color effect produced by the soils from any eminence. Standing in such a place on a day when the soils are thoroughly dry one notes that the general color of the landscape is a pale grayish brown, except where exposed rocks of different colors vary the conditions. Where the parent rocks of the mature soils are red this gray-brown color will be found only in the top inch or two of the soil profile, but where the color of the parent material is of a more sober hue, it extends to a greater depth. A composite profile description in outline form will serve to illustrate the characteristics of the desert soils of northwestern Wyoming. These soils have been designated as group 1 and the other groups will be taken up in order.

Group 1.—Pale gray-brown desert soils (8)

Depth of Horizon	*Description*
0– 1 inch A₁	Pale gray-brown "crust and mulch" Crust one-fourth to three-fourths inch thick, quite hard when dry, and filled with small ovate vesicles one-sixteenth inch in diameter Mulch of very fine, loose angular soil aggregates when texture is heavy, and single grain structure when soil is light textured.
1– 6 inches A₂	Light grayish to yellowish brown slightly laminated soil of medium texture.
6– 9 inches B₁	Compact, cloddy, yellowish brown soil of slightly heavier texture than above horizon. Most compact in profile ·
9–18 inches –40 inches B₂	This is the horizon of lime accumulation and as indicated varies greatly in thickness. It occurs as vertical streamers of light gray to nearly white crypto-crystalline lime deposit alternating with yellowish brown, limy soil. The proportion of lime decreases rapidly in the lower part of this horizon and none is visible below it Very commonly there is a deposit of white or pinkish crystalline or crypto-crystalline gypsum interspersed with the upper layers of the parent material beneath the lime horizon Soluble salts are often found below the gypsum
18–40 inches down C	The parent material may be rounded gravels of various igneous, metamorphic, or sedimentary rocks, or it may be red, yellowish brown, or gray sandstone. Where the sandstones are red their color persists in the horizons which underlie the crust and mulch. Pebbles and larger stones throughout the profile are almost invariably coated with lime, either entirely or on their under sides This lime is usually more or less indurated. On the high older terraces lime is rapidly accumulating along the weathering cracks in the stones. This is especially true of the basic igneous gravels. In the C horizon the gravels and sandstones retain most of their original characteristics, showing only a slight amount of weathering.

When the composite profile described in the foregoing is exposed in a road cut or along a stream channel, several things of interest are noticeable. In the first place it forms a series of fairly distinct columns which are noticeably

resistant to weathering. These columns are most noticeable in the lower A and B horizons. When thoroughly dried they are very hard and difficult to break up. The lime accumulation is soft and friable when moist but it often becomes slightly indurated when dry. Another striking characteristic of the profile is that a test with dilute hydrochloric acid gives a very strong effervescence from the surface downward. This is undoubtedly due to the fact that rainfall is insufficient to carry the lime down to the lower from the upper horizons as fast as carbonation takes place in the soil.

In numerous small flats or slight depressions there are usually small areas of "slick spots." In these places the soil has characteristics similar to the normal types but in a much more pronounced form. The columns are more clearly cut and harder to destroy; the lime accumulation is usually dense and there is a much greater accumulation of gypsum in the lower horizons. As soon as these lands are irrigated they become "puddled" and a heavy accumulation of soluble salts appears at the surface unless ditch or tile drains are provided.

The shales of this part of the country, as mentioned in the foregoing, so interfere with weathering as to prevent the maturing of the soils derived from them. Some samples taken in these shale soils showed that although the total depth of the solum was only 6 or 8 inches, the same series of horizons could be found as in the soils described above. This type of profile might be said to be dwarfed rather than immature in the stricter sense of the word.

The vegetative cover on the mature desert soils is usually quite light. It consists chiefly of two species of plants; namely, sagebrush (*Artemisia tridentata*) and salt sage or Winter fat (*Eurotia lanata*). The following table gives some of the less abundant plants and the nature of the soils with which they are associated.

PLANTS		SOILS
Common name	Botanical name	
Rabbit brush	*Chrysothamnus* (several)	Very sandy soils
Shadscale	*Atriplex confertifolia*	"Slick spots"
Greasewood	*Sarcobatus vermiculatus*	Salty spots (poorly drained)
Prickly pear	*Opuntia*	Medium and light textured soils
Grama grass	*Bouteloua oligostachya*	Normal types
Needle grass	*Stipa*	Normal types
Fleabane	*Erigeron*	Normal types
Pentstemon	*Pentstemen nividus* Dougl.	Residual uplands
Paint brush	*Castilleja angustifolia*	
	Castilleja buffumi Nels.	Normal types

There are many others.

These plants are not all confined to the soil types with which they are listed but are more abundant on those types and rarer on the others. However the shadscale and greasewood seldom spread beyond the slick and salty soils to which they are respectively adapted.
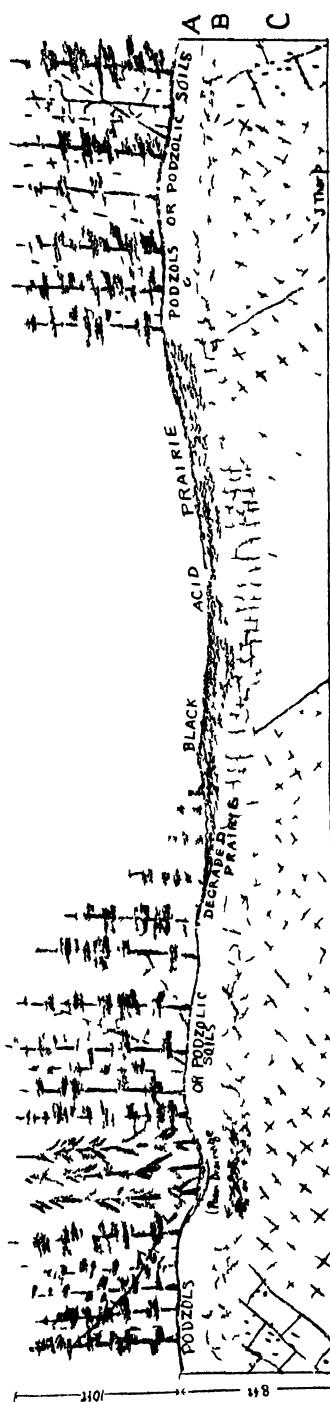
FIG 5 RELATIONSHIPS OF SOILS TO FORESTS AND PRAIRIES IN YELLOWSTONE AND IN BIG HORN MOUNTAINS
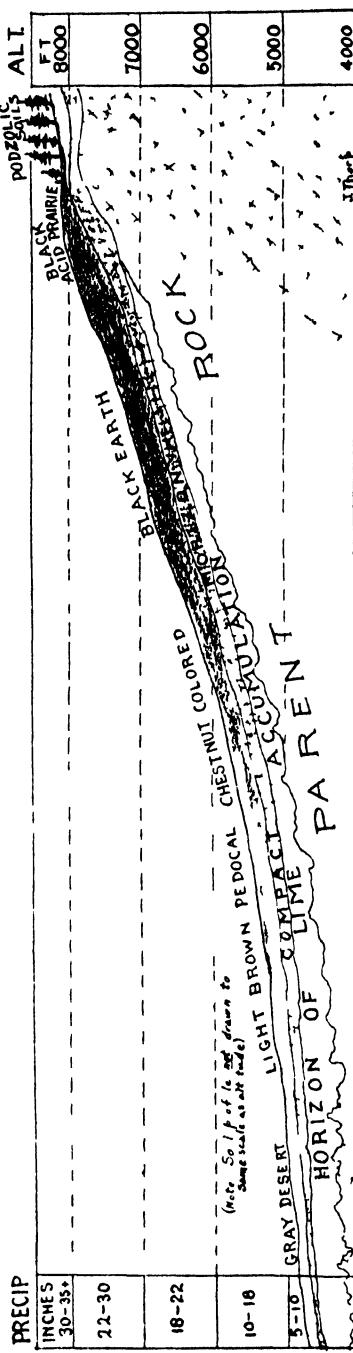
FIG 6 GRADATION OF SOIL PROFILE FROM DESERT TO HUMID MOUNTAIN TOP—WEST SLOPE OF BIG HORNS

Near the edges of the desert, where the rainfall is slightly greater, there is an increase in the amount of vegetative cover. The proportions of grasses and flowers increase and the salt sage gradually disappears. The sagebrush, however, persists and is very abundant in the moister regions of the mountain slopes.

Group 2.—Light brown soils

If we travel into the mountains eastward or westward from Big Horn Basin and are careful to follow smooth slopes where the soil has been undisturbed for a long period of time we find that the color of the A horizon of the soil changes very rapidly as progressively moister regions are reached. The first noticeable color change is to a light brown instead of gray. Tests with acid show little or no effervescence in the A horizon but Soiltex tests indicate that the pH is well over 7. The slightly compact or heavy horizon which is always found in this region just above the lime accumulation shows effervescence with HCl as do the horizons beneath. The lower horizons differ but little from those in the desert. Areas of this soil may be found near Hart Mountain, near the northwestern edge of the Basin, and on the lower slopes of the Big Horns near Hyattville and Shell. The vegetation on this type of soil has a much greater percentage of grasses and but very little salt sage. Sagebrush, however, is usually abundant.

Group 3.—Chestnut brown soils

Still higher on the mountain sides the A horizon becomes thicker, dark brown, and the pH value is about 7. The heavy layer in this type is thicker also and is cloddy and columnar. It contains a film of brown colloidal material spread over the surfaces of the clods. The depth to the lime accumulation is still greater than in the soils of group 2. Grasses and sagebrush constitute the principal vegetation.

Group 4.—Black earths or chernosem

. In this group the A horizon is from 12 to 15 inches thick, is laminated in the upper part and structureless in the lower part, and is neutral to slightly acid in reaction. The heavy layer is thicker than that in group 3. It is somewhat granular in structure and the granules are coated with brown colloidal material from the A horizon. The horizon of lime accumulation is thinner than in group 3. This soil supports a vegetation similar to group 3 except that it is more abundant.

Group 5.—Acid prairie soils

On the higher mountain tops where the vegetation is grass instead of timber and where the rainfall is over 20 inches the soil is a black or brown acid prairie. There is a laminated black soil about 1 foot deep underlaid by a yellowish brown, compact, granular horizon in which the soil aggregates are coated with brown organic colloids from the above horizon. At a depth of about $2\frac{1}{2}$ or

3 feet is the rotten rock material which constitutes the C horizon. The vegetation is chiefly grasses of various species with some Artemisia and a great profusion of wild flowers including lupine, Castilleja, Delphinium, Dodecatheon, and Myosotis. This soil in general strongly resembles the acid prairie soils of Iowa and Illinois.

Group 6.—Podzolic soils

The sixth group of soils exists under the same rainfall conditions as the soils of group 5, the only different factor in their environment being that of vegetation. This group of soils is always found with a heavy forest covering constituted chiefly of Lodgepole pine (*Pinus murrayana*) with some alpine fir (*Abies subalpina*) at high altitudes. Englemann spruce (*Picea englemannii*) is quite common in some parts of the mountains, particularly in moist areas. In many places the timber cover is so heavy that there is little undergrowth. However, where the trees are less plentiful there are some small shrubs and many bright flowers, one of the showiest of which is *Castilleja lauta*.

The soils of this group were developed under an annual rainfall of from 20 to 35 inches. Since they are at a high altitude where the average annual temperature is low, the "soil climate" is moister than one would expect from the absolute amount of the precipitation.

The sixth group of soils probably belongs with Glinka's podzols or podzolic soils, as a brief description of one of them will show. This sample was taken in the Big Horn Mountains in a thick grove of lodgepole pine and spruce.

DEPTH	HORIZON	DESCRIPTION
inches		
$0-\frac{1}{2}$	A_0	Raw humus of rotted pine needles, roots, wood, etc.
$\frac{1}{2}-1\frac{1}{2}$	A_1	Dark brown structureless loam
$1\frac{1}{2}-6$	A_2	Ashy gray structureless loam
$6-12$	B_1	Pale yellowish, heavy gravelly loam streaked with dark-colored material from above
$12-26$	B_2	Reddish yellow and yellow mottled gravelly loam, less heavy than B_1
$26-40$	C_1	Yellow mottled with reddish yellow rotten granite fragments

The entire profile is strongly acid in reaction, as tested with Soiltex. In several places it was observed that the forest was gradually encroaching on the adjacent prairie. In these places the black topsoil of the prairie was deep just outside the edge of the timber, slightly shallower where there were scattered young conifers, noticeably thin where the forest was becoming more dense, and entirely lacking where the original stand of heavy timber was reached. This transition takes place within a distance of from 50 to 100 yards. In places where the edge of the forest has evidently been stationary for a long period of time the transition from the podzolic soil to the black prairie takes place within a distance of 8 or 10 feet.

In endeavoring to correlate the findings of this investigation with the work of Marbut and of the Russian soil scientists, the writer recognizes the possibility of making errors in judgment. The reader should bear this in mind when considering the material presented in the following:

Proposed correlation of the soil groups of northern Wyoming with the great soil groups in the United States and Europe

WYOMING SOILS (J THORP)	SOILS OF U. S (C F MARBUT)	SOILS OF EUROPE (K GLINKA)
Group 1	Gray desert	Gray desert
Group 2	Brown pedocal	Brown (light chestnut)
Group 3	Dark brown pedocal	Chestnut colored
Group 4	Black earth	Chernosem
Group 5	Prairie	Not listed
Group 6	Podzolic	Podzolic

The mountainous character of much of the country and the differences in vegetation have caused minor differences between some of the Wyoming soil groups and their corresponding broad groups elsewhere in the world. Groups 1 and 2 and perhaps 3 more closely resemble their corresponding groups elsewhere than do groups 4 and 5. The podzolic soils of the Big Horns and of Yellowstone Park were not separated into gray-brown forest soils and podzols because no satisfactory method was found for locating division lines.

The major banding of soil groups in northern Wyoming around the Big Horn Basin has been described. A similar condition prevails east of the Big Horns, but group 1 is missing. Group 5 is found on the mountain tops but groups 2, 3 and 4 occupy the foothills and plains to the east. Group 4 is limited in extent to a narrow strip of land close to the foot of the mountains. Group 3 extends in tongues to a few miles east of Sheridan, and group 2 extends many miles eastward to the Black Hills region.

Undoubtedly these belts are found on the rolling plains instead of on the mountain slopes because the rainfall decreases less rapidly with the altitude on the east side of the mountains than it does on the west side. Sheridan has an annual precipitation of 14.43 inches as opposed to 5.96 inches at Basin, which occupies a similar physiographic position in the Big Horn Basin (9). Although there are no weather stations at the foot of the mountains south and west of Sheridan it is the opinion of the Weather Bureau men at Sheridan and of the writer, based on observation, that the rainfall increases rapidly between Sheridan and the foot of the mountains. Corresponding to this probable increase in rainfall there is a decided darkening of the A horizon in the soil. Farmers consulted testified to a greater rainfall in those parts where the mature soils are dark colored.

Another banding of soils which corresponds strikingly with the climatic zones is to be found in northern Yellowstone Park. An examination of the precipitation map of Yellowstone Park (fig. 3) reveals an area of low annual

fall around the lower reaches of the Yellowstone River and its tributaries. Because of the lack of time no soil samples were taken here but careful observations were made. Near the north edge of the park along the Yellowstone River there is a strip of group 2. Farther up the river and its tributary, the Lamar, members of groups 3 and 4 were successively observed. Elsewhere in the park, where the precipitation is heavier, groups 5 and 6 were observed and sampled.

SOIL REACTION

A number of pH tests of several of the samples were made[2] and these were supplemented by field tests made with Soiltex. In table 1 are given the approximate average pH values for the three principal horizons of each group of soils.

TABLE 1

pH values of northern Wyoming soils

SOIL GROUP	HORIZON A	HORIZON B	HORIZON C
1	8 55	9 22	8 25
2	8 5*	9 3*	8 0*
3	6 5–7 0*	9 0*	8 0*
4	6 0–7 0*	9 0*	8 0*
5	6 2	6 5	7 0
6	4 9	5 7	6 1

* Values determined by Soiltex tests; others determined by hydrogen electrode or quinhydrone methods

CONCLUSIONS

Detailed observations seem to warrant the following conclusions regarding the effects of vegetation and climate upon soil profiles in northern and northwestern Wyoming:

The presence or absence of lime in the soil, as determinable by ordinary field observations, depends upon the humidity of the soil as determined by the rainfall combined with the average annual temperature   Where the rainfall is light, lime forms in the soil more rapidly than it can be leached out by percolating waters, and it accumulates in a horizon which lies at the depth of average moisture penetration.   As the rainfall increases, the depth to this horizon of lime accumulation increases until under a rainfall of approximately 20 inches it disappears altogether insofar as can be determined by simple tests with acid

Gypsum and salt accumulations disappear sooner than the lime under the conditions mentioned in the foregoing.

The thickness of the heavy subsoil or upper B horizon increases with the increase in rainfall, other things being equal. This is the horizon of maximum illuviation.

The color of the soil is determined largely by the proportion of grasses in the vegetation, e g , the more grass, the darker the soil   Of course the amount of grass is largely dependent upon the rainfall in turn.

[2] Tests made by Ernest Bailey of the Bureau of Chemistry and Soils.

Under like conditions of rainfall and parent material a forest vegetation causes the formation of an altogether different soil from that formed under a prairie vegetation.

Topsoils in this region have a pH value of 6.5 or more when the precipitation is less than 20 inches, and less than 7.0 when it is 20 inches or more. The pH values are lower in forested soils than in prairie soils under the same rainfall conditions. In the pedocals the pH is highnest in the horizon of maximum lime accumulation, whereas in the acid prairie and forest soil it is highest in the C horizon.

SUMMARY

This article deals with the effects of vegetation and climate upon soil profiles in northern and northwestern Wyoming. Differences in climate are due to the great variation in elevation, whereas differences in vegetation are closely allied with those of climate. The soils have been grouped into six great classes based upon important profile differences. Soils are pale gray-brown on the desert and, with the exception of group 6, become progressively darker with the increase in altitude and rainfall. The sixth group, associated with heavy conifer forests, is light colored. The first four groups belong to the pedocals (soils having horizons of lime accumulation). They are separated from one another chiefly on the basis of the darkness of the color of the A horizons. Groups 5 and 6 are both in humid zones but differ greatly in profile characteristics. This difference is due to the great difference in vegetative cover. Group 5 has a heavy sod of grasses and flowers with no trees, whereas group 6 has a heavy growth of conifers. Parent materials are frequently the same under both groups.

APPENDIX

The appended table gives certain essential relationships between soils and precipitation and vegetation in this region.

SOIL GROUP	TOPSOIL COLOR	PRECIPITATION	VEGETATION*
		inches	
1	Pale gray-brown	5–10	Salt sage, sagebrush, rabbit brush, needle grass, grama grass, fleabane, pentstemon, prickly pear, paint brush, shadscale
2	Light brown	10–15	Sagebrush, salt sage, grama and other grasses, lupine, larkspur, paintbrush, pentstemon, Mariposa lily wild buckwheat, some juniper, loco, and Gaillardia
3	Chestnut brown	15–18	Same as above with no salt sage and with more sagebrush and a heavier sod
4	Nearly black	18–20	More grasses and sagebrush than above
5	Black or brown	20–40	Little sagebrush; heavy sod of grasses, lupine, pentstemon, paintbrush, Gaillardia, harebell, forget-me-not, shooting star, etc.
6	Ashy gray	20–40	Lodgepole pine, Douglas fir, Englemann spruce, ground juniper, lupine, paint brush, harebell, gentian (moist spots), and others

* Common names,— see below for botanical names

*List of plants identified**

COMMON NAMES	BOTANICAL NAMES
Fleabane	*Erigeron*
Forget-me-not	*Myosotis alpestris*
Gaillardia	*Gaillardia aristata*
Gentian	*Gentiana serrata and others*
Grama grass	*Bouteloua oligostachya*
Greasewood	*Sarcobatus vermiculatus*
Ground juniper	*Juniperus communis sibirica*
Harebell	*Campanula rotundifolia*
Indian paint brush	*Castillija angustifolia*, *C. buffumi* Nels. (on soils 1 and 2), *C palescens*, *C. linariaefolia* (on soils 3 and 4); *C. lutea* Heller (soil 5); *C. lauta* Nels., and *C. confusa* Greene (soil 6). There are several others
Juniper	*Juniperus scopulorum*
Larkspur	*Delphinium geyeri* Greene and others
Lupine	*Lupinus* (a large number of species not differentiated by the writer)
Mariposa lily	*Calochortus Nuttallii* and *C. Gunnisonii*
Needle grass	*Stipa* (specific name not determined)
Pentstemon	*Pentstemon nitidus* Dougl (soil 1); *P cyaneus* Pennell, and *P. Caryi* Pennell (soils 2, 3, and 4); *P. procerus* Dougl , *P. procerus pulvereus* Pennell, *P pseudoprocerus* Rydb , *P fruticosus* (Pursh), (on soils 3, 4 and 5) There are several others in this region
Prickly pear	*Opuntia*
Rabbit brush	Chiefly *Chrysothamnus dumosa*
Sagebrush	Chiefly *artemisia tridentata* with a number of less common species
Salt sage or winter fat	*Eurotia lanata*
Shadscale	*Atriplex confertifolia*
Shooting star	*Dodecatheon pauciflorum*
Wild buckwheat	*Erigonium* (species?)

* Dr. F. W. Pennell, Curator of Plants, Philadelphia Academy of Natural Sciences and Dr. A. Nelson of the University of Wyoming furnished most of the plant identifications.

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PLATE 1

THE GRAY-BROWN DESERT SOILS OF BIG HORN BASIN

FIG. 1 Typical vegetation on gray-brown desert soils of Big Horn Basin. In foreground are clumps of salt sage (*Euroiia lanata*) and some grama grass (*Bouteloua oligostacha*). In the middle ground is a colony of sagebrush (*Artemisia tridentata*). Taken near Basin, Wyo.

FIG. 2. Showing how the gray-brown desert soils of Big Horn Basin stand in vertical columns where exposed by erosion. Taken near Lovell, Wyo.

FIGS. 1 AND 2

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PLATE 2

ACID PRAIRIE AND PODZOLIC SOILS IN THE BIG HORN MOUNTAINS

FIG 1   Showing open grassy lands and alpine fir thickets at 9,000 feet elevation in Big Horn Mountains.   Soils under timber are podzolic those with grass are brown acid prairie

FIG 2   Open prairie with strips of lodgepole pine forest at 8,500 feet elevation, Big Horn Mountains   The road in the foreground exposes the black surface soil of the acid prairie Soils in the forested areas are podzolic
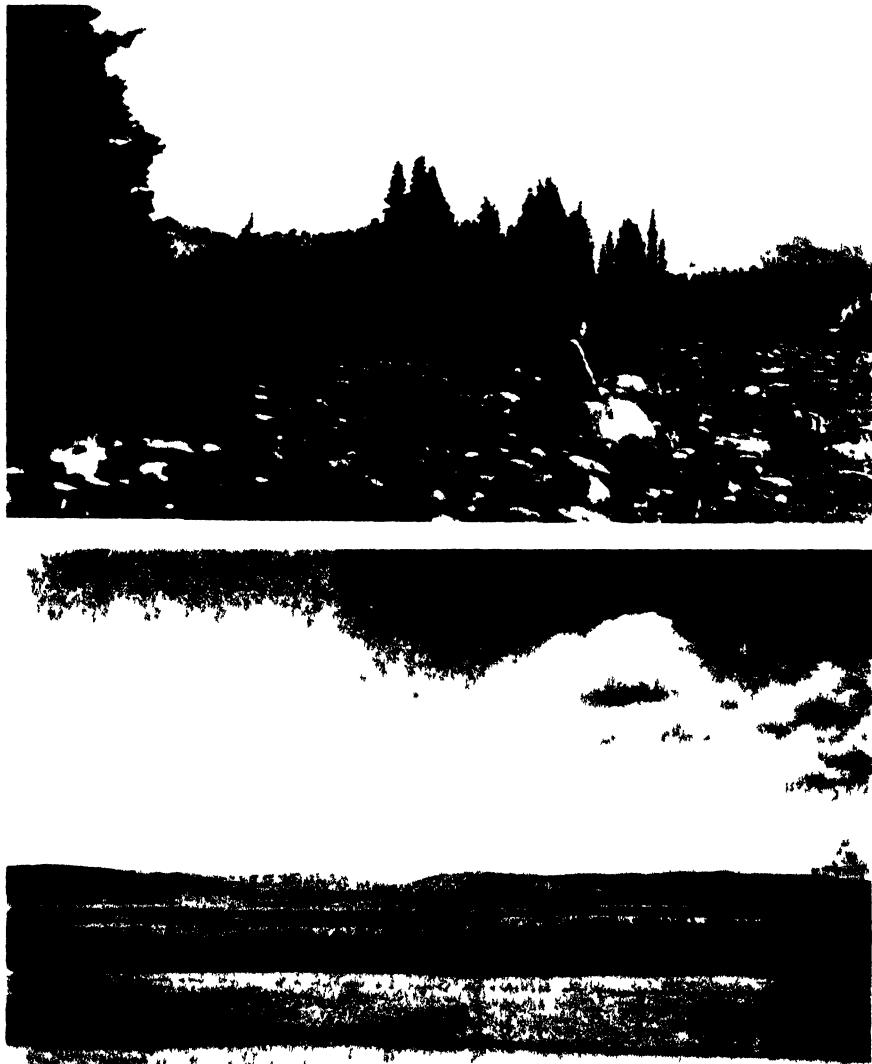
FIGS. 1 AND 2

SOIL PROFILE STUDIES: III. THE PROCESS OF PODZOLIZATION

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In connection with the soils profile studies conducted at this station, the process of podzolization as evident in the soils of New Jersey has been investigated. In view of the general interest in the theories of the process, it was deemed advisable to present first a discussion of the subject.

HISTORICAL

Long before the true nature of podzols was established the bleached layer just a little below the surface of the soils was observed and noted. Almost a century ago Sprengel (45) described what we know now as podzol soils.

Scandinavian and German foresters and geologists also noted and described podzols. In the excellent work of Muller (33) mention is made of the views held by some Danish geologists, especially those expressed by Selmer, that the ash-gray to white horizon in some of the forest soils in Scandinavia is not formed in situ, but it has been brought in by the winds or laid down by water. They considered the bleached horizon as an independent geologic stratum. Muller and later Ramann (39) refuted the idea about the white layer being an independent geologic formation, but they did not explain either the origin or the process responsible for the "white" sand formation. Not until the Dokuchaev school of soil science revealed the genetic relationship of the soil horizons in the profile was it possible to elucidate the mode of formation of the bleached layer in the forest soils of the temperature region and the North. As a matter of fact Dokuchaev (7, 8, 9) began his soil studies on the podzols of the Smolensk region in Russia. But it was Sibirtzev (41) who separated these soils into a special type.

Ever since the classical researches of Dokuchaev the podzol soils have been studied in great detail and a wealth of information has accumulated on the process of podzolization. Close to 200 papers are cited bibliographically by Glinka (18) just on the soils in the podzol zone within the boundaries of the U. S. S. R. In recent years the literature on podzols has been augmented by a series of papers in German and English, especially since 1914 when the work of Glinka (19) became available to the German reading workers and later to the English readers through the translation by Marbut (19).

For our purpose it will suffice to mention the work of just a few Russian

investigators as well as the more important work of others whose investigations have a direct bearing on the subject under discussion.

<center>THEORIES ON THE PROCESS OF PODZOLIZATION</center>

In all the studies on podzol soils the process of podzolization has been linked with the soil organic acids and organic matter in general. Ramann (39), Dokuchaev (9), Sibirtzev (41), Glinka (18) in his early work, and a number of other investigators stressed the rôle of the organic acids in the process of podzolization. They were under the influence of the German workers Mulder (31, 32) and Detmer (5), who subdivided the soil humus into neutral and slowly moving ulmin and humin and into slightly soluble humic acids and easily soluble crenic and apocrenic acids.

Dobrovol'skii (6) summarized the work on podzols up to the year 1900. He systematized the data in tabular form and on the basis of these and his own work he concluded the following:

The upper horizons are high in total SiO_2 because the other constituents are leached out In general podzols are rich in Fe and poor in Ca. The P content of the A horizon is lower than that of the B. When wet the bleached horizon is a sticky, smeary mass, which upon drying hardens and when crushed becomes powdery, silty like, especially in the loams.

In his work with the podzols Dobrovol'skii attempted to differentiate the organic matter in the respective horizons. An $(NH_4)_2CO_3$ extract from the A_1 horizon gave a dark brown solution; from the A_2 bleached horizon, a red-yellow solution. When neutralized with acid, humic acid settled out from A_1 but not from A_2. The filtrate from A_1 resembled in color the extract from A_2. Both the filtrate from A_1 and the extract from A_2 upon addition of $CuSO_4$ gave a precipitate of a dirty green color (crenic and apocrenic acids). In the bleached A_2 layer there is little apocrenic acid; it is mostly the less oxidized stage—the crenic acid. There is no humic acid in this horizon, which is therefore white or ash-gray.

Bogoslovskii (4) traced the southern limits of the podzol process in Western Europe. He points out that the podzolization process might be extended to the yellow and red soils of the Mediterranian where it is not so intense as north of that zone.

Williams (55), whose views on the processes of soil formation are at some variance with the orthodox Dokuchaev school of soil science, applied the modern knowledge of base exchange and other replacement reactions to the scheme on the differential rôle of the various organic acids—especially crenic and apocrenic—in the process of podzolization.

Because of a definite similarity between the views of Williams and Dobrovol'skii, cited in the foregoing, they are presented at this point.

The process as pictured by Williams[2] is as follows: The crenic acid formed

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[2] Williams rarely presents experimental data to prove his points. His treatises are primarily of a scholastic, speculative, and philosophical nature. In some respects they are extremely interesting.

in the decomposition process of the forest litter reacts first of all with the carbonates of calcium, liberating CO_2 and forming calcium crenate, which is soluble. Not until all the lime has disappeared does the crenic acid react with the other elements of the parent material. Iron and manganese are attacked next, forming crenates of these elements, which are also soluble and are therefore carried downward. The loss of iron and manganese causes the whitish or gray color. After leaching out of the sesquioxides, which according to Williams (55, p. 116) are

. . always present in the parent material in a very fine state of division, the kaolin or the hydrate of alumino-silicic acid ($H_2Al_2Si_2O_8 \cdot nH_2O$) is disintegrated by the crenic acid. Under the influence of this acid, free sols of silicic acid are separated out, whereas the aluminum forms the crenate. Other metals of the same group—iron, chromium, and manganese—also form crenates.

The aluminum crenate is leached downward. Some of the silica sol moves downward in colloidal state, some turns into gel, which loses water and in an amorphous state fills the interspaces in the parent material imparting to it the white color and a structureless condition

Such is the complexity of phenomena which results in the formation of the podzolized horizon in the upper layer of the parent material.

The products of the podzol process of soil formation are classified by Williams into two groups:

1. Mineral salt of all the elements of plant nutrients formed in the decomposition of the forest litter and

2. The salts of the organic acids rich in nitrogen—the crenates of Ca, Fe, Mn, and Al

In the podzol horizon neither bacteria—because of the acid medium due to the crenic acid—nor fungi—because of the crenic acid which is a waste product of the fungi—may develop. It thus becomes a dead horizon where only mineral reactions will take place.

Kossovich (26) in discussing the processes of soil formation sums up the podzol process as follows:

It takes place under conditions of sufficient or excess of moisture. The organic acids together with the carbonic, nitric, and perhaps sulfuric acid act on the mineral constituents and dissolve the bases. Finely divided organic substances in colloidal state are also active. During periods of saturation ferrous salts might be produced which leach out. Primarily, however, aerobic conditions exist and the iron probably moves as the sol protected by the organic colloids. Silica is usually left behind. As a result of the leaching of bases the horizon of eluviation is acid. The horizon of illuviation becomes enriched with electrolytes which are responsible for the precipitation of the iron and aluminum, which serve as cementing material for the ortstein formation or incrustations. Some of the fine clay particles are also caught in their downward movement in the horizon of incrustations or ortstein which is identified as the B horizon. As a result, this horizon becomes compact even if the parent material is not of clay origin.

The processes described are responsible for the formation of a grayish-white horizon just below the humus accumulative horizon. It is poor in bases, iron, aluminum, and colloidal substances in general, and acid in reaction. In soils poor in organic matter or in sandy soil, this layer is practically free from organic matter, poor in bacteria, and rich in fungi mycelium. This process prevails in the northern part of the temperate zone. At the northern and southern limits of this zone the so-called intrazonal gradations of podzols are found. Within the

zone one finds regions of true podzols, strong podzolization, medium podzolization, and light podzolization.

Among the more important investigations on the process of podzolization are those of Zakharov (56). He analyzes the process of podzolization with reference to the moisture régime as influenced by the macro and micro-relief of the region. He outlines the development of the various types of podzols from a slightly podzolized soil to a peat podzol and finally to a peat-gley. His conclusions are similar to those of Tumin, whose work is discussed presently and who presented diagramatically the idea of relief influence (fig. 4).

Tumin (50) separates the podzol zone into five belts. In the center of the zone there is a true podzol belt. To the north and south of it there are the two belts of podzolized soils, and north and south of these there are the two belts of weakly or slightly podzolized soils. The differences in the analogous belts—

TABLE 1

Variations in the degree of podzolization due to the mechanical composition of parent material

PORTION OF THE ZONE	BELTS	SOILS ON LOAMS	SOILS ON SANDY LOAMS	SOILS ON CLAY LOAMS	LOWER BLEACHED HORIZON (BELOW B)
Northwestern portion	5	Weakly	Podzolized	Podzols	Present
	4	podzolized	Podzols	Podzolized	
Central portion	3	Podzols	Podzolized	Weakly podzolized	Is disappearing
Southeastern portion	2	Podzolized	Weakly podzolized	Weakly podzolized	Absent
	1	Weakly podzolized	Weakly podzolized	Weakly podzolized	

north and south of the central true podzol belt—consist of the following: in the northern belts we find more organic matter and a higher reducing activity (due to more frequent saturation) than in the southern belts.[3] There is another morphological distinction between the analogous northern and southern belts. In the soils of the northern belts there is a bleached whitish horizon below B; none such is found in the southern belts.

In figures 1 and 2, reproduced from Tumin (50, p. 324), a podzolized and a true podzol soil with a bleached horizon (Ba_2) below B are represented.

Tumin points out that the fall in the intensity of podzolization both in the northern and southern belts is conditioned by the two principal climatic factors: rainfall and temperature.

The fall in degree of podzolization in the south-eastern direction takes place under conditions of lower rainfall and higher temperature; in the north-western direction—under conditions of higher rainfall, lower temperature, and decreased aeration.

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[3] In reality the belts do not run north and south, but northwest and southeast.

According to Tumin (50)

The description outlined on the distribution of podzols, podzolized, and weakly podzolized soils is true for loams on a level plain topography.   Otherwise the morphology of each belt in the podzol zone is closely related to the relief, mechanical and chemical composition of the parent material.

In table 1, Tumin presents

. . . . a crude scheme of the distribution of the podzols, podzolized, and weakly podzolized soils in connection with the variations in the mechanical composition of the parent material.

Changes in the degree of podzolization in connection with fluctuations in relief take place in the direction from a higher elevation to depressions.   The degree of podzolization will change in the same manner as it does in a given parent material as we advance to the northwest.   In the southeastern zone (first belt) the podzolization on loams, sandy loams, and sands increases in the direction of the depressions, i.e. in this belt true podzols are to be encountered in the depressions only.

In the central portion of the zone (third belt) podzolization weakens on loams in depressions and increases on the sandy loams and sands.   It is perfectly clear that with the transition into depressions the deoxidation process and accumulation of organic matter (peat formation) increase in the soil horizons.   With the approach toward the depressions the depth of some of the horizons increases.   A new sub-horizon A_1', darker in color than A_1 appears, it replaces A_1 in the depressions and wherever peat is formed it is gradually covered by the peat layer A_0 which finally replaces it.   [*See* fig. 3.]

A graphic illustration of the aforesaid is given in figure 4, reproduced from Tumin (50, p. 328).   It shows how A_0 gradually replaces A_1 with the change in microrelief.

"The presence of carbonates in the parent material tends to depress podzolization."   Under such conditions an intrazonal morphological type known as "rendzina" is formed.

The amount of humus in the soils of the podzol type varies as follows at the extreme northwest and southwest there is an increase in the humus content; from these extremes toward the central portion of the zone the humus content decreases.

In another paper Tumin (49) differentiates the processes of podzolization and leaching of bases.   The latter is true also for other soil processes.   Podzolization is intimately related with the light colored humus substances—crenic acid—and the dark colored—humic acid.   It is the predomination of the crenic acid over the humic acid in the A_2 horizon of the podzol profile that determines the podzolization effects.   .

Tumin (49) disagrees with Sibirtzev (41, p. 257), who, in his pioneer work on podzols, pointed out the parallelism between podzolization and the separation of SiO_2 in the bleached horizon.   To Tumin the accumulation of the light colored humus is the characteristic feature of podzolization.

Glinka (18) has shown experimentally—by igniting soil from the bleached horizon—the presence of the light colored humus substances: upon ignition the material darkens, which indicates carbonization of organic matter.

Neustruev (35) in discussing the relationship of the organic acids to the process of podzolization justly remarks: "No one has proved the presence of crenic acid and in general its existence and properties are thus far hypothetical."

In an attempt to establish the conditions of iron and aluminum precipitation in podzol soils, Aarnio (/) conducted a series of experiments on the rôle of humus in these reactions. He obtained ammonia humus extracts from two peats, dialyzed them for 5 weeks and then mixed definite quantities of the extracts with variable quantities of iron and aluminum sols. From the data on the iron and aluminum coagulates, ratios were obtained for the amount of humus (by weight) necessary to coagulate one unit (by weight) of Fe_2O_3 or Al_2O_3.

One humus extract gave a ratio ranging from 1 Fe_2O_3:2.79 humus to 1 Fe_2O_3:0.90 humus; the second humus extract gave a ratio 1 Fe_2O_3:2.46 humus to 1 Fe_2O_3:0.82 humus. For aluminum the ratios with the respective humus extracts were: 1 Al_2O_3:30.12 humus to 1 Al_2O_3:1.20 humus and 1 Al_2O_3:26.62 humus to 1 Al_2O_3:5.32 humus. This would indicate that colloidal iron under the influence of humus moves deeper into the soil profile than aluminum.

Aarnio found that colloidal SiO_2 and the anions SO_4 and PO_4 are also instrumental in precipitating the iron sols.

In a second paper dealing with the same subject Aarnio (2) presents more data. He shows how the ratios vary with the type of humus source. He points out that in soils with a high humus content there is a tendency for a low $Fe(OH)_3$ and a high $Al(OH)_3$ content.

Smirnov (44) attempted to demonstrate experimentally the process of podzolization. Tubes, 5 cm. in diameter, were filled with soil material from a podzol sandy soil. First the material from the C horizon was placed in the tubes followed by A_2 and A_1. The B horizon was left out in order not to hinder percolation. The percolates consisted of: distilled water, distilled water saturated with CO_2, and a weak ammonia (1:100) solution. From time to time 50–60-cc. portions were titrated. The H_2O percolates were yellowish brown and acid in reaction, requiring for neutralization 0.25 to 0.5 cc. of 0.01 N NaOH. The CO_2 percolates were colorless and alkaline for the first 3 days after which they became acid. The ammonia extract was acid, dark brown, and continuous leaching with ammonia produced under the A_2 material a ring resembling ortstein formation.

A thorough discussion of the process of podzolization is presented by Glinka. His many investigations and observations are presented in the two compilations of his life work (16, 18). His views may be summarized as follows:

Fundamentally the process of podzolization is nothing more than the carrying away of the mobile sols of humus deprived of its calcium from the surface horizons. These sols also serve as a protective agent for the downward movement of the fine mineral suspensions. All of these mobile substances are deposited in the B horizon.

The white coating which gives the characteristic appearance to the bleached horizon consists of finely powdered quartz sand which remains on the surface of clay lumps from which the finest clay particles and the hydrates of iron oxide have been washed away.   Glinka (18, p. 336) states:

It has been proposed that the white coating is a result of the splitting off of the silicic acid in the process of silicate decomposition by the humic acids.   If that were the case one would expect an appreciable quantity of silica soluble in alkali and alkali carbonates.   Experiments, however, show that in podzols there is no more soluble silica than if the alkali would react with finely powdered quartz.

Besides that, if the silica in the podzolized horizon represents the residue from the decomposition of the silicates, one would expect to find in the B horizon considerable quantities of aluminum hydroxide, which is not borne out by the experiments of Gemmerling.   The small quantities of free hydrates of aluminum found by Gemmerling in the ortstein might come from the ash constituents of the plants.

Gedroiz (13) interprets the process of podzolization in the light of base exchange reactions.   Whenever conditions are favorable for the replacement of the cations in the soil complex (mineral or organic) capable of base exchange with hydrogen-ions, podzolization takes place.   Such conditions prevail in the climatic regions of moderate rainfall where the percolating waters exclude the accumulation of soluble salts.

TABLE 2

Water extract analyses in per cent of weight of soil

SOIL	DRY RESIDUE		SiO_2	$Al_2O_3 + Fe_2O_3$	CaO	HUMIS (BY TITRATION)
	Ordinary drying	Igniting				
Original chernozem.................	0 0575	0 0195	0 0095	0	0 0107	0 0360
Chernozem saturated with H	0 1140	0 0504	0 0430	0 0055	0	0 0605

Pure water upon dissociation gives at 22°C., 10^{-7} gm. of hydrogen ions per liter.   In the presence of carbonic acid, the amount of hydrogen ions appreciably increases since the dissociation constant of this acid is 3×10^{-7}.   A still greater increase in the concentration of hydrogen ions takes place in the presence of organic acids, which are the products of organic matter decomposition. The processes of nitrification and sulfur oxidation introduce into the soil solution the highly ionized nitric and sulfuric acids.   Thus the concentration of hydrogen ions in the soil solution may reach at some time a magnitude of considerable moment.

The energy of replacement of hydrogen is higher than that of Ca, Mg, K, and Na.   It means that the replacing power of the H ion when compared with an equimolecular or ionic quantum of Ca, Mg, K, Na, or a combination of these in solution, is greatest.   On the other hand, the total concentration of the other cations—Ca, Mg, K, Na—either individually or in combination, might reach a quantum whereby the total energy of replacement of them becomes high enough to suppress the activity of the H ions.

Gedroiz (13, p. 66) points out that

. . . . water circulating in the soil when saturated with CO_2 under pressure of one atmosphere will contain 10^{-5} gm. of H ions per liter; under the same conditions the solubility of $CaCO_3$ will bring into solution about 5×10^{-1} gm. of Ca per liter, i.e. its concentration will be 50,000 times higher than that of the H ions. It is perfectly clear that in the presence of calcium and magnesium carbonates in the soil, or any other more soluble salts, the processes which take place in the parent material and in the soils will not tend to produce an unsaturated complex.

Thus the formation of an unsaturated soil complex and hence of a soil unsaturated with bases is possible only when almost all of the easily and difficultly soluble salts have been leached out and carried down to a depth from which the capillary rise of moisture cannot bring them to the surface horizons.

The replacement of the bases with hydrogen and the formation of an unsaturated complex (organic and mineral) are not the only result of the action of water on the soil. To quote Gedroiz (13, p. 68):

Water exerts on the soil alumino-silicates (and humates) not only the influence of base replacement; it also partly disintegrates them at the same time, converting them into more simple compounds and partly decomposing them into the simple oxides of iron, aluminum, and silica. The action of water on the aluminum silicates and humates unsaturated with bases is a good deal more effective than on the silicates which have not reached that stage yet

To give some idea of how water is more active on soils unsaturated with bases, analyses are presented in table 2. A 100-gm. portion of natural chernozem saturated with Ca and Mg, and a 100-gm portion of the same chernozem from which the bases had been removed and replaced with hydrogen were each mixed with 500 cc. of water. Both mixtures were kept for 3 days and the water extract was analyzed.

Thus the soil loses not only the bases but also the colloidally dispersed humus and the complex mineral compounds which decompose into silicic acid and hydrates of the oxides of aluminum and iron. In this way the processes of degradation and podzolization lead, on the one hand, to the formation in the soil of insoluble compounds unsaturated with bases and, on the other hand, to their decomposition.

According to Gedroiz (13, p. 69) the process of podzolization consists of two stages:

First stage. The hydrogen ion of the water replaces the absorbed bases in the absorbing complex which is unprotected by the presence of minute quantities of simple salts against the entry of these ions.

Second stage. The portion of the complex unsaturated with bases begins to be energetically disintegrated by the water. The colloidally dispersed humates are carried downward and the aluminum silicate portion of the unsaturated complex is decomposed into silica and the oxides of iron and aluminum.

Thus the views of Gedroiz on the process of podzolization differ somewhat from those of Glinka inasmuch as the latter does not admit that in this process the aluminum and iron silicates decompose completely leaving behind most of the silica, while the iron and aluminum move downward. In the scheme of Gedroiz the decomposition process is one of the essential features. Besides

that, Gedroiz postulates the formation of aluminum silicates anew in the B horizon through the mutual coagulation of the positive Al and negative SiO_2 sols.

Ortstein and concretion formation

There is a general agreement that in the process of podzolization a translocation of mineral and organic substances—crystalline and primarily colloidal—takes place in the soil profile.

Eluviation and its complement illuviation find expression in the morphological features and chemical composition of the soil profile. The horizon of eluviation—A—becomes impoverished of bases, sesquioxides, colloidal mineral and humus particles, and its complex capable of base exchange becomes unsaturated. It has no definite structure. The horizon of illuviation becomes enriched with the substances from the A horizon and attains a definite morphological appearance, chemical composition, and physical make-up. Its general features are common to all groups of podzols: it is darker in color, more compact, has structure, contains more clay and electrolytes, and is higher in base exchange capacity than the A_2 horizon.

In certain important details, however, the B horizon differs in various podzols. One of the variable elements is the ortstein formation which is identified with the B horizon in the podzol zone. Not all podzols, however, possess ortstein or even concretions, and these new formations are not therefore to be looked upon as inherent to the process of podzolization.

Ortstein formation has been known for a long time. Müller (33) distinguished three fundamental groups: (a) ortstein due to washing in of fine particles, (b) orstein formed by absorption of humus and iron, (c) ortstein of a concretional origin. In the last group Müller places also a type of bog iron formation which should not be classed with the ortstein because ortstein is a formation of the downward movement of substances and bog iron is a product of the rising waters.

Ramann (40) identifies podzols with pan formation. According to him (40, p. 69):

> The pan is a layer of the soil cemented together by humus and since the podzols are preponderately sandy soils, this is a humus sandstone. Soft, easily friable pans may be termed "earthy pans" (Orterde).

Ramann (39, p. 167) differentiates three forms of ortstein: (a) "branderde" which forms a mass rich in organic matter but not cemented together, (b) ortstein hard as a rock, dark brown to black, with an average content of organic matter; it is encountered in the northern part of Germany, (c) a dark brown ortstein, very hard, containing a small amount of organic matter; it is usually very thick and is overlain by a softer and lighter layer.

Warrington (54) points out that humic acids dissolve out iron, which moves downward and precipitates on sand, forming iron pan.

An extensive study of podzols and ortstein formation has been made by Tamm (48). He considers ortstein as unweathered material cemented with colloids, primarily iron (limonite), and humus. Ortsteins rich in humus are soft, whereas those rich in iron are hard.

Chemical analyses of ortstein and orterde are presented by Lundblad (28) in connection with his investigations of the podzols in the coniferous forests and degraded brown earths in the beech forests of southern Sweden. He points out that the horizon of accumulation with orterde formation in the podzols contains a higher amount of gel than any horizon in the profile. Another fact brought out in his work is that the inorganic gel content of ortstein formation is relatively small when compared with the organic colloids.

A discussion of the work of Tamm and Lundblad as well as of the more important investigations on the ortstein formation is given by Stremme (47). He quotes van Bemmelen, who designated ortstein "as a cementing of the sand particles with colloidal humus, silicic acid, aluminum and iron oxide complexes, and some clay fractions."

According to Aarnio (2) the process of ortstein formation is to be identified with the movement of the sesquioxides and humus from the surface horizons into the lower ones. The iron and aluminum move as sols protected by the humus, by the silicic acid sols, as well as by the cations Ca, Mg, K, Na, and the anions SO_4, PO_4, and CO_3. If there is little humus, iron ortstein, poor in humus, forms. With large amounts of humus, humus ortstein, poor in iron, forms. The precipitation of sesquioxides is homogeneous in sandy soils. In loam and clay soils they precipitate in cracks and tracks of roots.

The relation between the parent material and ortstein formation has been investigated by Münst (34) and Helbig (22).

Stremme (47, p. 134) discusses the influence of ortstein formation and podzolization on forest growth and the relationships between podzolization and type of vegetation. He quotes Hazard (21), who shows that under certain conditions ortstein is an asset to the physical conditions of the soil. He also quotes Hausrath (20) in this connection. Tamm (48) concludes that podzol and ortstein formations are of no great significance from the forestry standpoint.

Emeis (10) attempted to approach the subject of ortstein formation experimentally. He filled several cylinders with quartz sand, added the various constituents which make up the ortstein, and percolated daily a humus extract through that. He succeeded in forming a humus-iron ortstein by the reaction of the sesquioxides, humus, and lime.

Filatov (11) passed colloidal solutions of iron hydroxide through columns of quartz sand with intermediate thin layers of fine quartz (0.05–0.001 mm.). He found that only the fine quartz became colored with iron. Intermediate layers of finely ground minerals (orthoclase, biotite) exhibited a still greater retentive power for the iron.

A discussion of hardpan or ortstein is presented by Hilgard (23, p. 184). He correlates its formation with the deposition of iron, clay, and humus.

Skeen (42), who defines hardpan as "that stratum found at varying depths below the surface composed of 'clays' and sands more or less cemented by precipitated Fe, Al, and sometimes organic compounds," correlated hardpan formation with the hydrogen-ion concentration of the medium. Whenever the pH of the clay soil he was working with was 4.8, hardpan was found. He conducted experiments showing how $Fe(OH)_3$ precipitated on kaolin at that particular pH.

In a second paper Skeen (43) advances a theory on the formation of hardpan in acid clay soils. If the reaction of the upper layers of the soil is acid enough to bring into solution Fe and Al, hardpan might form.

The limited observations of Skeen should not have been generalized. As a matter of fact almost all the soils in the podzol zone, except where the parent material is limestone, are sufficiently acid to dissolve some Fe and Al and still not all podzols, even of clay texture, have an ortstein or hardpan layer. It is well established now that podzols and ortstein formation do not run parallel.

Summarizing the work of Western European and Russian investigators on ortstein formation, Glinka (18, p. 325) points out that

. . . horizon A_2 becomes impoverished of bases and sesquioxides, when compared with the parent material, and the ortstein becomes enriched with the sesquioxides and manganese, but not always with bases, except magnesium. Ortstein decomposes more rapidly than the parent material in HCl solution. The solubility of alumina in ortstein increases very markedly.

Poluinov (36, 37) investigated mineralogically and chemically the new formations in ortstein. He found a mineral of the polygorskite type. It is a magnesium aluminum silicate and apparently this is the source of magnesium which is so frequently encountered in ortstein.

Gemmerling (14) demonstrated the presence of free aluminum hydroxide in ortstein concretions, which is in accord with the theory of Gedroiz (13) that in the podzol soils some portion of the aluminum silicate nucleus is decomposed.

At times the ortstein formation appears in the profile as narrow winding bands of a dark brown iron-like color. They were studied by Visotzkii (53), who designated them as pseudofibers. They are encountered primarily in weakly podzolized soils.

A review of the problem of pan formation is given by Jones and Willcox (25). They discuss some of the earlier theories and present some experimental evidence on the process of pan formation. Extractions were made with dilute solutions of tartaric or oxalic acid on soils from several podzol profiles. The extract from the A horizon was filtered, a portion sampled for analysis and the rest used for the extraction of the soil from the B horizon. Again the extract was filtered, sampled for analysis, and the remainder used for the extraction of the soil from the C horizon. The data obtained show that the sesquioxides go into solution in the A horizon and precipitate in the B horizon. It is suggested that the sesquioxides enter

. . . . into the electro-negative portion of the molecule, since soil organic acids consist largely of hydroxy acids and these acids dissolve the sesquioxides to form salts of the type $Fe_2R_3 \cdot Fe_2O_3$. These compounds are then leached through the soil in solution and are ultimately precipitated as basic salts, thus giving rise to a zone of sesquioxide accumulation and finally to a pan

Another type of ortstein formation is the concretions which appear at the bottom of the A_2 and on the top of the B horizon. They contain variable amounts of humus, iron, and sometimes manganese. The humus content in the concretions is greater than that in the surrounding soil material. Usually concretions are found in clay podzols.

Some pedologists link the process of podzolization with ortstein and concretion formation. Tumin (50) does not share this view. According to him:

Ortstein is encountered on sandy parent materials only, on loams there is no ortstein, but concretions (iron and manganese) in the form of grains or complexes of grains. And even on sands ortstein is not always found. The same might be said about concretions on loams. Thus ortstein and concretions do not accompany all podzolized soils, except several of them which form under certain conditions.

It is of interest that ortstein is always found at the boundary between horizons A and B. This is not true for concretions, which are to be found in all horizons. In the southeastern portion of the zone (on the sands) ortstein is found also in the depressions. In the north western portion ortstein gradually appears as we go from the deep depressions to the level topography

In general the facts of ortstein and concretion deposition indicate that they are formed under conditions of change from deoxidation to oxidation processes in the soil.

GLEY FORMATION

In Russia the popular understanding of gley, according to Visotzkii (52), is "a more or less compact, sticky loam or clay parent material, which is not, however, as sticky as the usual loam or clay, frequently with a more or less clearly pronounced light greenish blue tinge."

Gley formation is found in marshes and under conditions of a high water table. The gley horizon is generally water-logged, except when the water table recedes. In this horizon, therefore, anaerobic conditions prevail, favor reducing reactions, and minimize leaching effects. Besides, the ground waters rise by capillary forces and enrich the gley horizon with bases, imparting to it an alkaline reaction.

The simultaneous downward and upward movements of the substances in solution bring about a unique condition with respect to the iron. The anaerobic state is inducive to reducing reactions and the insoluble iron compounds become soluble and move downward. At the same time the rise of substances in solution by the capillary forces brings back some iron to a point where it might come in contact with the air and precipitate. We thus find an ocherous layer on the top of the gley horizon. This is probably the chief factor in the process of bog iron formation. While the upper layer of the gley horizon becomes enriched with iron, the lower layer becomes impoverished of iron. It

is in this layer of the gley horizon where we find the mottling effects with the characteristic gray bluish-green tinge of the material.

In many respects, the process of ochre formation is similar to that of the rising of salts in alkali soil formation. This has been pointed out by Visotzkii (52), who was probably the first one to study gley formation.

Within the podzol zone, especially in the northern belt of it adjoining the forest tundra belt, gley formation is not uncommon. This does not exclude
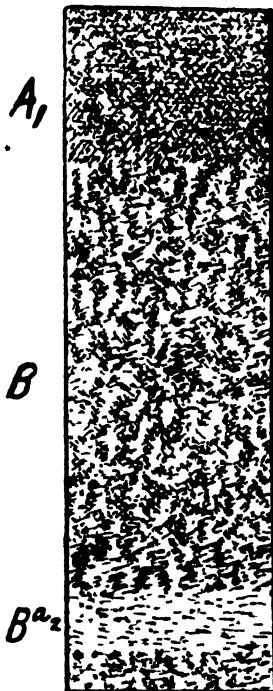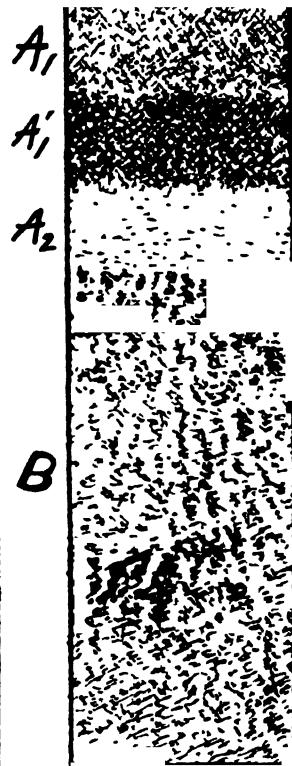
FIG. 1   FIG. 2   FIG 3

FIG 1 WEAKLY PODOZOLIZED SOIL WITH A BLEACHED HORIZON BELOW B
FIG. 2 A PODZOL WITH A BLEACHED HORIZON BELOW B
FIG. 3. A PODZOL WITH A DARK COLORED SUBHORIZON A_1'

the existence of gley formation in other soil zones, since it is not a climatogenic formation but a hydrogenic. Indeed Visotzkii (52) points out the presence of gley formation in the southeastern Precaspian steppe.

Gley formation has been studied primarily in the podzol zone and has therefore been associated with this zone. Whenever gley formation is found on a podzol soil, it is the C or B horizons which undergo the respective changes. The A_2 horizon remains intact, i.e. the rising waters do not reach this horizon.

Frosterus (12) in his work on the podzols with clay as parent material and a high water table discusses the question of gley.

An extensive study of soils with a gley horizon in the podzol zone has been made under the direction of the late Glinka by Zavalishin (57).   His observations are summed up as follows:

The gley horizon appears in the form of a sandy or clay material of a light-gray or gray color with a bluish, blue, or sky-blue tinge.   The color is not uniform, it depends on the intensity of gleying and on the mechanical composition of the material.   Usually the gray blue background is mottled with large red spots and veins.   Usually these spots are associated with the cracks and root paths and they are more frequent in the clay varieties.   The spots around the roots may be of two kinds: if the material is not strongly gleyed and there is
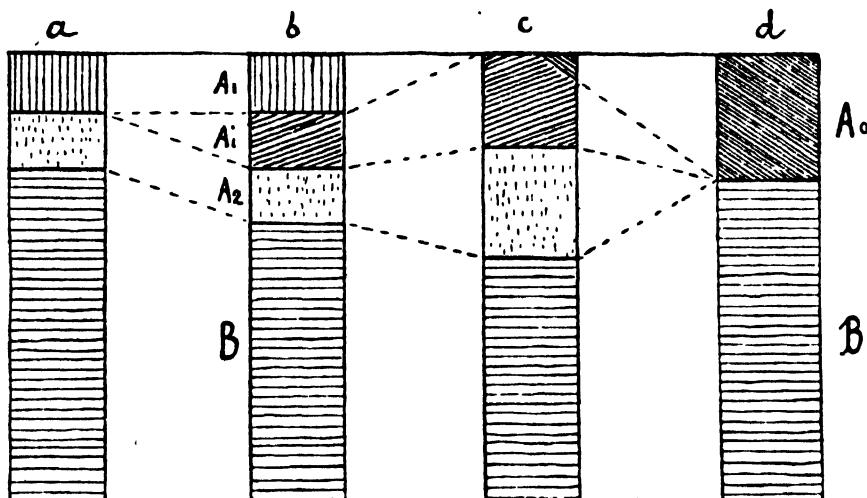
Fig. 4. A Diagrammatic Presentation of the Influence of Micro-relief on the Gradual Replacement of the A_1 Horizon by the A_1' Subhorizon and Finally by the A_0 Horizon (Peat Formation)

a, level place; b, slight depression; c, a slightly deeper depression; d, a peat depression.

decomposed organic substance in the root path, then we have a light gray-bluish gley formation with a reddish band on the outside.   If, on the other hand, the gleying has proceeded very far and the root path is nothing more than a tube through which air passes, then a red ring forms around it on a light-gray bluish background.   When the gleying is very strong then the parent material is of a homogeneous gray-blue coloration without any spots or veins. Gley horizons, especially sandy, at times resemble podzols; the bluish tinge and the red spots identify it, however, as gley.

Usually the gley horizons are without structure, more or less compact, sticky, smeary, and appear to be more clayey than the adjoining parent material.   A suspension of gley formation does not settle out and it is highly dispersed.

The humus content is higher in the upper layer of the gley horizon than in the lower. The solubility of the humus increases with an increase in gleying.   With that, the reducing property of the gley increases and this in turn influences the reducing reactions with respect to the iron compounds.

Zavalishin presents a lot of data which seem to indicate that the gley horizon, designated as G, either approaches neutrality or is neutral or alkaline. With respect to saturation with bases the gley horizon differs but little from the parent material. It varies depending on the composition of the ground waters. One thing is certain: it contains a higher content of replaceable magnesium and slightly less calcium. There is also a slight increase in silica. The presence of reduced iron compounds in the gley has also been demonstrated.

From what has been said, it may be inferred that *gley formation on podzols indicates the termination of the podzol process of soil formation and the inauguration of the marsh or tundra types of soil formation.*

FOREST-GRAY AND BROWN EARTHS

Within the borders of the large climatogenic zone of podzols two other types, or rather subtypes, have been recognized: (a) the gray-forest and (b) the brown earths, known as the "Braunerde" of Ramann.

Forest-gray soils.—The forest-gray soils develop under conditions of deciduous forests. In a way they are a transition type between the chernozem and the true podzols. A striking characteristic of the forest-gray soil profile is the gray color of the A horizon which turns into brownish with depth. Chemically these soils differ but little from the true podzols. Both are acid with an increase in the intensity of acidity from the surface down to horizon B where the acidity decreases. The base exchange capacity is lowest in A_2, the latter being the poorest in colloids. With depth the capacity for base exchange increases because of the illuviation of the colloids. In the process of its formation some of the silicates in the surface horizon suffer disintegration and decomposition whereby some silica separates out on the nut-like structural units of these soils. (Dokuchaev in his time described the gray forest soils as "nutty").

Whenever the forests advance on the steppe, the soil becomes degraded. It loses its original morphology; a translocation of the humus and mineral substances takes place in a similar manner as in the podzols and the soils approach the morphology, composition, and make-up of the forest-gray soils. The podzol process of soil formation sets in as soon as the forest cover is capable of keeping the surface horizon moist and thereby favoring the leaching reactions by the percolating rain waters.

A discussion on the process of podzolization in the forest-gray soils is presented by Il'in (24). He takes up the question raised by Glinka (18) about the process of podzolization being secondary in the forest-gray soils. He links the secondary nature of the podzolization process on the forest-gray soils with the concept of degradation.

A comprehensive review on the genesis and occurrence of forest-gray soils is presented by Tyurin (51) and Levchenko (27).

Brown earths or "Braunerde" of Ramann.—According to Ramann (40)

. . . . the brown earths develop under the influence of a temperate climate which fluctuates greatly; some years are wet and others dry, so that the leaching of the soil is very much greater

at some times, very much less at others. The rainfall is not sufficient during the warm season to form seepage water in soils which are covered with vegetation. In warm and dry years slightly arid conditions prevail, so that the effects of the ascent of the ground water are seen. This is mainly evidenced by the soil water being enriched in calcium carbonate, though deposits of calcium carbonate in appreciable quantities are rare, or are only found in soils having an abundant water supply as in the loess. Leaching preponderates in most of the brown earths; the soluble salts and the earthly carbonates are washed out, while phosphates and the sesquioxides are retained in the soil.   . . .

In no other soil formation does the parent rock exercise such a large influence as in the brown earths. . . . . The soil has normally a neutral or slightly alkaline reaction; hence readily dispersed humus bodies are not found.

A series of brown earth profiles are described by Glinka (15). He considers them as a variety in the podzol zone, and as a condition for the formation of the "Braunerde" he lays down the prerequisite of lime carbonate in the parent material.

In another place Glinka (17) states:

All the participants of the excursion [in connection with the soil conference in Hungary] who happened to study the brown earths in their respective countries have agreed with me that the brown earths examined by us represent one of the varieties of the podzol type of soil formation on carbonate loams. It is to be noted that the brown soils in most cases contain carbonates in the lower horizon.

In still another place Glinka (18, p. 343) expresses the idea that these soils are of a transition type; "the brown earths of Western Europe represent, so to speak, the last stage of the podzolized type of weathering; they are transitory to the more southern yellow and red earths."

Some brown earths in Crimea have been described by Antipov-Karataev and his collaborators (3). They found no effervescence in the B horizon. Stebut (46) has recently contributed to the problem of brown earths.

Prasolov (38) agrees with Glinka that the brown earths are a transition stage from yellow and red soils of the southern regions into podzols.

It is of interest to note in connection with the process of brown earth formation that a number of investigators who described brown earths—Tamm (48, 48a), Lundblad (28), and Prasolov (38)—invariably associated them with beech forests. A glance at the analyses of the ash constituents of beech forest litter shows that the per cent of ash is high in beech (5.57 per cent in beech against 1.46 per cent for pine) and the ash contains high amounts of CaO [2.46 per cent against 0.59 per cent for pine; the analyses are taken from Glinka's (18 p. 30) work]. All of the CaO and the other bases complete a definite cycle which in its turn has an influence on the process of soil formation. It comes to the plant from the horizons of root distribution and the greatest portion is redeposited in the humus accumulative horizon A_0 to become mineralized and leached out. With the high amounts of ash constituents and especially Ca in beech litter, the replenishment of leached bases is high and thus a hindrance to the process of podzolization.

GENERAL DISCUSSION

With Tumin's (50) presentation of the process of podzolization as the central idea, a puzzling element appears, namely, that, according to him, the process of podzolization is more pronounced in the soils with a heavier texture than in those with a lighter texture. It is to be remembered that Tumin's contention, based on his wide experience and numerous investigations is applicable to the central belt of the podzol zone. He points out that the loam soils in this belt are true podzols whereas the sandy loams are merely podzolized. No explanation is offered by Tumin for such a behavior. It would seem that on the lighter soils the percolation effects should be more clearly expressed than on the heavier soils. On the other hand, one might expect quantitatively a more intensive reactivity in the heavier soils, which contain a larger supply of internal factors of podzolization, especially organic matter which furnishes the decomposition products of microbial and chemical activity, such as carbonic, nitric, sulfuric, and other inorganic and organic acids and colloids.

It seems that the process of podzolization is to a large extent intimately related to the speed of decomposition and quantity of organic matter. A too rapid destruction of the organic matter depresses the reactivity efficiency of the split products, since under such conditions the organic acids produced are usually decomposed to the so-called mineralized state. The result is that the rôle of the organic acids is reduced to a minimum.

It is very probable that such are the dynamic forces in the process of laterization whereby the residual mineralized cations (especially the monovalent as well as the H ions) link in some manner with the SiO_3 anion from the Al and Fe silicate complexes and move downward leaving behind the sesquioxides. Since the organic complexes are completely mineralized the sesquioxides have no humates to combine with. In soils undergoing the process of podzolization some of the bases and of the sesquioxides combine with the humates and move downward to enter again into a reaction (depending on a number of factors, especially the pH) with some SiO_3, forming new Al and Fe silicate complexes. The work of Mattson (29, 30) at this station on the behavior of the anions with respect to the sesquioxides in acid and alkaline media, or rather on the acid and alkaline side of the isoelectric precipitates, promises to discover the fundamental principles of this process.

In the belts north of the true podzol belt the organic matter, as a rule, does not undergo such rapid decomposition and does not therefore mineralize so fast. Under such conditions the percolation element of podzolization dominates the process and, of course, the lighter soils are more thoroughly and efficiently leached. The result is that in these belts the lighter soils become more rapidly podzolized than the heavier soils.

The considerations presented open the question of the rôle of organic matter in the process of podzolization from an entirely new angle. We are not concerned so much now with empirical studies of the early investigators on the

relative importance of the hypothetical crenic and apocrenic, and humic acids in the soil profile. We are concerned in learning the condtions under which the various organic substances which form humates behave in one way or another. The work of Mattson (29, 30) on the isoelectric precipitates of the sesquioxides under various conditions of pH and electrolyte content and the anion exchange relation with respect to the humates offers an approach to the study of organic matter distribution in the soil profile of the podzol zone. A study of a large number of cases of carefully sampled profiles would be necessary before the existing conditions in the soil might be correlated with Mattson's theoretical deductions.

From this point of view a survey of the organic matter distribution in the profile of the soils along the Atlantic slope from Maine to Florida would give us valuable information on the geographic distribution of the process of podzolization. It would take in the true podzols, the podzolized soils, and the transitional brown earths (perhaps also some forest-gray soils) blending into the yellow earths, red earths, and finally laterites.

Some of the points presented in this paper will be taken up and illustrated in a forthcoming paper dealing with the work done on some of the podzol and podzolized soils of New Jersey.

SUMMARY

A historical review of the subject is given.

The theories on the process of podzolization as presented by some of the important Russian investigators and others have been reviewed and differences pointed out.

The relation of ortstein or pan formation to the process of podzolization and the mode of its formation and occurrence have been discussed.

Gley formation as related to the process of podzolization has been discussed. It is pointed out that gley formation on podzols indicates the termination of the podzol type of soil formation and the inauguration of the marsh or tundra type.

The question of the relation of forest-gray soil to podzols has been touched upon.

The position of brown earths or "Braunerde" in the zone of podzolization, their relation to the type of forest vegetation, and the process involved have been discussed and analyzed.

A somewhat new aspect of the rôle of organic matter in the process of podzolization has been suggested and discussed. It seems that the speed of decomposition and mineralization of organic matter and the relation of the humates in the anion exchange reactions as worked out by Mattson with the isoelectric precipitates have an important bearing on the variations in the process of podzolization throughout the different belts of the podzol zone.

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BOOK REVIEWS

Handbuch der Pflanzenernährung und Düngerlehre, vol. II. By F. HONCAMP. Julius Springer, Berlin, 1931. Pp. xii + 919, figs. 285.

The entire volume is devoted to the subject of manures and fertilizers and their use. In dealing with so extensive a field of science and practice the editor and his associates were evidently compelled to condense much of the material treated under nine general topics. These consist of manures and manuring; natural manures; artificial manures; the use of artificial manures; the manuring of cultivated crops; the use of manures in forestry; the manuring of marsh and heath soils; the manuring of ponds; and manuring, manures, and plant protection.

The term "manures" is used here in its broadest definition to include animal waste products (livestock manures), industrial by-products (tankage, cotton-seed meal, basic slag, etc.) and chemical compounds manufactured specifically for increasing the supply of available plant food for crops. In the United States, on the other hand, the terms "manures" and "fertilizers" are employed to describe animal manures on the one hand, and industrial by-products (organic and inorganic) and synthetic compounds on the other.

The historical review of the subject is short, but adequate. The natural manures as dealt with by the authors include human waste products, green manures, composts, and stable and barnyard manure. Under the general topic *artificial manures* we find a discussion of potash, lime, phosphates, nitrates and ammonium salts, cyanamide, urea, and of salts and mixtures containing two or more of the major plant nutrients. The organic and inorganic waste and by-products used in commercial fertilizers are also described more or less fully. Some space is devoted to a discussion of *plant stimulants*.

The fertilization of specific crops in the groups of root and tuber crops, cereals, legumes, oil and fiber crops, vegetables, grapes and tree fruits, hops and tobacco is discussed within a space of about 200 pages. The fertilization of the soil in forest nurseries and in the forest itself offers much that is suggestive. No less interesting is the discussion of the methods employed toward increasing the supply of food for fish-culture. Materials containing lime, potash, phosphoric acid, and nitrogen are all considered as means toward increasing in ponds and lakes the supply of algae and of other food for fish. The use of chemical fertilizers in their relation to diseases, insect enemies, and parasites of crops; and likewise their value in combating weeds are dealt with more or less adequately. Altogether, the book is a distinct and valuable addition to our reference material on manures and fertilizers.

Die Schlämmanalyse. By HERMANN GESSNER with introduction by Georg Wiegner. Akademische Verlagsgesellschaft M.B.H., Leipzig, 1931. Pp. vii + 244, figs. 102, and 1 table.

This is the volume 10 of the series "Kolloidforschung in Einzeldarstellungen" founded by Zsigmondy and edited by Freundlich.

The author has rendered a real service in systematizing and in outlining in a helpful way the recent, as well as the older, information on soil colloids. After presenting the theoretical considerations on which the mechanical analysis of "fine earth" is based, he enters into a discussion of the various methods, apparatus, and devices employed for the purpose. His familiarity with the literature is apparent and his references are well chosen. In describing the procedures employed in the laboratory he again shows himself to be master of practice, as well as of theory. The book should prove to be a valuable aid to the teacher and a source of fruitful information for the student.

Entwicklung der Bodenkartierung Landwirtschaftlicher Betriebe und die Moglichkeiten Ihrer Praktischen Leistung. By W. TASCHENMACHER. Max Weg, Leipzig, 1930. Pp. 78, illus. 4, charts 1.

It is noted by the author that the mapping of farm land was not begun until the nineteenth century and that the first valuable soil map to become widely known was prepared in 1868. Soil classification and mapping acquired significance only in comparatively recent times and largely because of their direct relation to problems of soil management. The first 35 pages of the book are devoted to a discussion of different systems of soil classification, namely, those based on geology, agricultural chemistry, and internal soil characteristics. In part II of the book (pp. 44–67) the soils of the estate Krzyzanki, representing an area of 302 hectares, receive especial consideration from the soil management point of view. Part III is devoted to the mapping of soils of individual farms as a distinct aid toward their effective utilization agronomically, as well as economically.

Australian Rain-Forest Trees. By W. D. FRANCIS. Anthony James Cumming, Brisbane, 1929. Pp. xi + 347, 213 full-page, half-tone illustrations 25 text figures, 1 rainfall map.

We are told by the author that:

Rain forests consist of closely spaced, dense vegetation composed mostly of trees, shrubs, vines and herbaceous plants. Sometimes they are so dense that is difficult or impossible to penetrate them without the use of cutting tools   . In rain forests, especially in those of a luxuriant character, the trees harbour numerous plants known as epiphytes. The commonest epiphytes in rain forests are ferns, orchids, and lichens. . . Very prominent among the conditions which accompany rain forests in Australia is a high rainfall. The writer has remarked that the luxuriant rain-forest areas of Queensland have an average annual rainfall approximating or exceeding 60 inches.

The reader will find in the book a wealth of carefully arranged information on the nature and distribution of rain forests and their relationship to rainfall, geology, and soils. The topics specifically dealt with include the height and size of trees, buttresses, flanged stems, corrugated woody cylinders, the bark, wood, and leaves of Australian rain-forest trees. The families are briefly described and a key is suggested as a means of facilitating identification. Pages 42–340 are given over to descriptions and illustrations of the species.

J G. LIPMAN.

FIELD METHOD FOR LIME REQUIREMENT OF SOILS[1]

R. H. BRAY AND E. E. DE TURK[2]

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The determination of the base-exchange capacities of the major Illinois soil types has brought out the fact that these soils vary greatly in this respect (1). The variation is so great that it makes it impossible to make an entirely satisfactory lime requirement recommendation based on any method which measures either directly or indirectly only the active hydrogen ions in the soil.

Since in any well-drained soil in a humid region the hydrogen-ion concentration may be considered to be a resultant of the degree of saturation of the soil base-exchange complexes with bases, it follows that the same degree of saturation of these complexes with bases in two different soils may give approximately the same hydrogen-ion concentration, and yet the amount of bases present in each and also the amount of bases necessary to saturate completely these soils may vary considerably. The two extremes in Illinois are represented on the one hand by sandy loams having a base-exchange capacity as low as 3.4 m.e.,[3] and on the other hand by the clay loams having base-exchange capacities as high as 37.3 m.e. Starting with any given degree of unsaturation, the clay loams would require approximately 10 times as much base to saturate them fully as the sandy loams require. To illustrate this, two samples have been selected from over 250 soils in which had been determined, first, the pH by the hydrogen electrode method; second, the total replaceable bases by a titration method (2); and, third, the base-exchange capacity by a modification of Kelley's method (6) in which $CaCl_2$ instead of $NH_4C_2H_3O_2$ was used as the replacing agent. From these data the degree of saturation was calculated by dividing the milli-equivalents of replaceable bases present by the base-exchange capacity and multiplying by 100.

The first soil of the two illustrative examples is a sample of brown silt loam, with a base-exchange capacity of 21.5 m.e. and 11.5 m.e. of replaceable bases present. The second is a sample of light gray silt loam on tight clay with a

[1] Contribution from the division of soil fertility, department of agronomy, University of Illinois, Urbana, Illinois Published with the approval of the director of the experiment station.

[2] Associate in soil survey analysis and chief in soil technology, respectively

[3] The abbreviation m.e. represents milligram-equivalents in 100 gm. of soil. For those who are accustomed to think in terms of field limestone requirements, it may be stated that 1 m.e. computed to $CaCO_3$ is equivalent to 1,000 pounds of $CaCO_3$ an acre of 2,000,000 pounds of soil.

base-exchange capacity of 10.9 m.e. and 5.7 m.e. of replaceable bases present. To bring the brown silt loam up to 80 per cent of saturation[4] will require 5,700 pounds of $CaCO_3$ an acre (2,000,000 pounds soil), whereas the light gray silt loam on tight clay will require only 3,000 pounds. The degree of saturation is 53 per cent in both cases, the pH of both soils is 5.4, and the colors produced by the thiocyanate test are almost identical. The base-exchange capacity, the total replaceable bases, and the calculated lime requirements are almost exactly double for the one soil as compared with the other, yet the degree of· saturation and the pH are the same.

As has been stated, these two samples were selected from more than 250, as ideal examples to show the non-conformity of the pH values with calculated
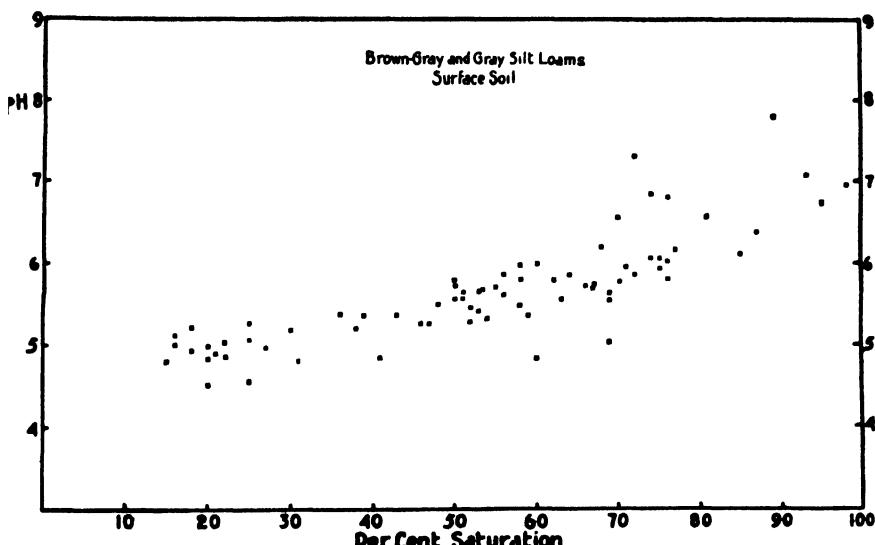
FIG. 1.—RELATION OF pH VALUES AND DEGREE OF BASE-SATURATION OF THE BASE-EXCHANGE CAPACITY IN 74 SAMPLES OF SURFACE SOIL

These samples represent 16 soil types, and base-exchange capacities ranging from 7 to 17 m.e.

lime requirement. A consideration of a large number of results gives a more accurate conception of the relation between these two values. Figure 1 shows the pH values and percentage of saturation of some surface (unlimed) soils, brown-gray silt loams, and gray silt loams, plotted against each other. The base-exchange capacities of these soils range from 7 to 17 m.e. These values as plotted form a curve. The breadth of the curve, however, indicates either

[4] Investigations in this laboratory have shown that satisfactory growth of sweet clover is obtained on many widely different soil types when the degree of saturation with replaceable bases is 70 to 80 per cent. The limestone necessary to raise the degree of saturation to the upper figure, 80 per cent, has therefore been chosen as representing the lime requirement of the soil.

that our present methods are not sufficiently refined or else that other factors which influence this relation are not being considered. A difference in the nature of the base-exchange complexes in the different soil types, or the presence of Ca or Mg salts of organic acids soluble in the salt leaching solution would, if present, tend to broaden the curve. It is found further that this curve is relatively flat below 60 per cent saturation but rises fairly abruptly at this point and at the same time becomes somewhat broader. Thus, the correlation between pH and degree of saturation of the base-exchange capacity is better in those soils which are more than 60 per cent saturated, than in less saturated soils.

The heavier silt loams and the clay loams have been omitted from figure 1 because certain samples among them show such a wide variation from the curve that it is evident that the aforementioned method of calculating the degree of saturation cannot be entirely relied upon for all soils. These are poorly drained types high in organic matter. They give an apparently high degree of saturation accompanied by a fairly low pH. Further studies on these soils have shown that they contain more acid than can be accounted for by the difference between the base exchange capacity and the replaceable bases present, as measured by the aforementioned methods.

From these relationships, it is obvious that there are two objections to the use of the hydrogen-ion concentration or any value depending upon it as a basis for limestone applications. One objection is the absence of a sufficiently close correlation between pH values and degree of saturation of the base-exchange capacity in soils which are less than 60 per cent saturated. The other objection is that the base-exchange capacities of soils vary greatly and unless this value is known in the case of an individual soil sample, the pH value gives no clue as to the amount of limestone needed to bring the degree of saturation up to any desired level. Morgan (10), in discussing pH and lime requirement, gives a review of the literature on this subject which indicates that a relation exists only on soils of the same type or closely related types. Where soils of such similar types are used the base-exchange capacities are very likely to be similar (1), a situation which would partially overcome the second objection stated, i.e., that the base-exchange capacities may vary greatly.

The measurement of all the acid present, or of all the base which the soil will absorb, would give a value entirely too high for a limestone recommendation. It is well known that the pH of the endpoint in titrating a weak acid against a relatively strong base is considerably above 6.5 to 7, which may be considered an endpoint for liming as far as satisfactory sweet clover growth is concerned. Figure 1 shows that this pH range in unlimed soils occurs at approximately 80 per cent saturation.

The average value for the limed plots on seven Illinois experiment fields on widely varying soil types which grow excellent sweet clover is 81 per cent saturation. Although sweet clover[5] will grow at pH values below 6.5 or at a

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[5] Sweet clover has been chosen as a reference crop partly because of its high sensitivity to acid soil conditions, and partly because of the importance of conditioning soils to grow this crop for soil improvement.

degree of saturation below 80 per cent, it has been found in greenhouse pot experiments[6] that the maximum crop was obtained only where the degree of saturation with bases had been brought to between 70 and 80 per cent. If a satisfactory condition for sweet clover growth can be obtained by increasing the degree of saturation to 80 per cent, one is hardly justified in making a limestone recommendation based on the total acid present, since with soils of high base capacity this extra 20 per cent would mean an increase of 3 or 4 tons of fine limestone, or 4 or 5 tons of screenings.

As a result of these conclusions, it was thought desirable to devise a method which would measure approximately the amount of limestone necessary to increase the degree of saturation of the soil with bases to 80 per cent. Such a method would be of practical importance as a means of measuring lime requirement. A method has been worked out in this laboratory using for its calibration the numerous soil samples on which the base-exchange capacity and degree of saturation have already been determined. This method is based on the following reasoning.

When a sample of acid soil is shaken in a salt solution, the base of the salt replaces part of the replaceable hydrogen. The resulting equilibrium is expressed by the following equilibrium equation:

$$KCl + H\,X \longleftarrow\!\!\!\!\!\!-\!\!\!\!\!\!-\!\!\!\!\!\!-\!\!\!\!\!\!- HCl + K\,X$$

where X represents the colloidal soil complex capable of the surface absorption of H ions and other cations. Now, if a small amount of base or base reacting salt such as Na_2CO_3 be added to the solution, the HCl will be neutralized until all the added base is used up and a new equilibrium will be established during which more K ions will replace more H ions on the colloidal surface. Since a part of the total amount of acid present will have been neutralized, the resulting new equilibrium will be one in which a smaller number of hydrogen ions exists in the solution, and consequently, the pH of the solution will be higher. As more and more base is added, the pH will continue to rise. Now if to any given soil whose base exchange capacity and amount of replaceable bases are known, just enough base is added to increase the degree of saturation to 80 per cent, the resulting solution should have a certain pH which will be for all practical purposes very similar for any soils so treated. Peats, mucks, and similar soils are not included in this discussion, although it is believed that the method as finally worked out can be used on them.

It was reasoned that if an indicator could be found whose color change comes at about this pH, one could then take a soil whose degree of saturation and base-exchange capacity are unknown, shake separate portions of it with a salt solution with varying known amounts of base, and determine what amount of base is necessary to bring about the desired change in color of the indicator.

[6] E. E. DeTurk and R. H. Bray, unpublished work.

Experiments have shown that when KCl is the salt used, and Na_2CO_3 is the base used, this endpoint can be determined on soils of varying base-exchange capacities and degrees of saturation by the use of bromthymol blue. The intermediate green to green-blue color of this indicator gives the endpoint for this determination. The procedure used was adopted after numerous trials with various salts, different indicators, and varying amounts of water.

This determination is, in effect, a titration method, but differs from the usual recommended methods [see review of methods by Clark and Collins (4)], in that the endpoint of the titration was chosen as a result of its agreement with an 80 per cent degree of saturation rather than selecting, for example, a pH of 7 for the endpoint because it represents neutrality.

In developing the method, the calibration was effected by using numerous soil samples whose base-exchange capacity, total replaceable bases, and pH were known. The soils used represented a wide range of types from black clay loams through brown silt loams to light sandy loams. A few samples of the darker soils whose pH did not seem to agree with the degree of saturation within the limits of the experimental curve were not used in the calibration, although the results on them obtained with the method as finally adopted have been included in table 1. This table gives for comparison the amounts of limestone necessary to reach an 80 per cent degree of saturation as calculated from previously determined base-exchange data and the amounts as found by the titration method. It will be noticed that the titration method has an advantage over the base-exchange method, since by the former a definite though small lime requirement is given for those soils whose calculated degree of saturation is high and yet which have a low pH. A low pH indicates unsaturation, even though it is not a quantitative measurement of the amount of limestone needed.

The general agreement between the lime requirement as calculated from the base-exchange data and that found by the titration method is fairly good except for the soils already mentioned as being subject to error in the base-exchange data. In fact, Chapman and Kelley (3) conclude that neither the replaceable base methods nor the base-exchange capacity determinations yield highly exact results, so that a difference of 1 m.e. corresponding to 1,000 pounds of $CaCO_3$ per acre would not be accounted a large error in any soil. The soils have been arranged in table 1 in order of increasing calculated requirements and arbitrarily separated into groups. For any one group the average lime requirement, as computed from the base-exchange data, is approximately the same as that found by the titration method. Excluding the first three groups containing neutral soils and most of those soils subject to error by the base-exchange method, there are only 5 cases in 61 in which the variation between the two methods is over 1 ton. Since the results of the titration method are likely to vary either above or below the calculated amount, it is considered that this method measures approximately the amount of bases necessary to raise the degree of saturation to 80 per cent.

TABLE 1

Comparison of the amount of limestone in pounds per acre necessary to raise the degree of saturation to 80 per cent as determined by calculation from base exchange data and by the direct determination by the Na_2CO_3 method

LABORATORY NUMBER	TYPE NUMBER*	BASES REQUIRED CALCULATED	BASES REQUIRED BY Na_2CO_3 METHOD	pH	THIOCYA-NATE†
		pounds	*pounds*		
12059	17	0	0	7 79	0
13058	14	0	0	7 07	0
11941	18	0	0	6 95	1
11976	43	0	0	6 85	0
13012	14	0	0	6 64	0
12096	56	0	0	6 60	0
	Average	0	0		
11931	18	0	430	6 72	0
11936	18	0	1,290	6 56	0
11914	41	0	860	6 43	0
11982	65	0	860	6 48	0
	Average	0	860		
12053	66	0	430	6 99	1
12018	66	0	430	6 63	1
11964	43	0	860	6 31	2
12029	66	0	1,290	6 28	2
11988	41	0	1,290	6 15	2
11293	43	0	430	6 11	3
12024	66	0	1,720	6 25	4
11958	43	0	1,290	5 88	4
11995	41	0	1,290	5 86	4
11970	43	0	1,290	5 64	4
11898	41	0	1,290	5 45	4
11908	41	400	860	6 16	2
12005	41	600	2,580	5 66	4
	Average .	77	1,158		
11300	127	1,000	1,290	6 07	2
11919	41	1,300	1,720	6 07	1
11314	46	1,300	2,580	5 95	3
12035	59	1,400	1,720	5.90	2
S6393	41	1,500	3,010	5 52	3
11892	41	1,500	3,440	5 29	5
11925	41	1,500	1,290	5.93	3
11307	127	1,500	1,720	5.87	3
12012	41	1,500	1,290	6.18	3
11807	11	1,700	2,150	5 04	4
11342	46	1,800	2,150	5.76	2
12069	18	1,800	3,010	5 84	3
	Average	1,483	2,114		

* Illinois soil survey type number.
† Thiocyanate readings reported on scale of 0 = no color; 5 = dark red.

TABLE 1—*Continued*

LABORATORY NUMBER	TYPE NUMBER*	BASES REQUIRED CALCULATED	BASES REQUIRED BY Na₂CO₃ METHOD	pH	THIOCYA-NATE†
		pounds	*pounds*		
11825	2	2,100	3,010	4 83	5
11180	112	2,100	2,580	5 69	4
13030	14	2,200	1,720	5 95	3
11335	124	2,300	2,580	5 73	5
13029	14	2,300	1,290	5 78	3
11876	14	2,400	1,290	5 97	4
11229	11	2,600	2,550	5 37	4
13036	14	2,600	2,150	5 56	4
13043	14	2,700	1,720	5 66	3
13037	14	2,800	1,720	5 52	4
11864	13	2,900	2,150	5 72	4
11222	12	2,900	3,010	5 37	3
11870	13	2,900	2,150	5 48	4
11257	11	3,000	3,870	5 41	5
	Average	2,557	2,271		
13024	14	3,200	2,150	5 26	4
11857	12	3,500	2,580	5 49	2
11250	11	3,500	5,160	5 20	4
13000	14	3,600	3,010	5 56	3
11349	127	3,800	2,150	5 79	3
11286	127	3,900	3,870	5 51	4
13070	14	4,000	2,510	5 25	3
	Average	3,643	3,061		
11145	113	4,200	3,870	5 32	3
13071	14	4,200	3,010	4 76	4
11243	12	4,400	5,590	5 20	4
11272	12	4,400	3,440	5 26	3
13044	14	4,400	4,300	5 14	4
11882	21	4,500	4,300	5 05	5
S6391	41	4,500	5,160	5 15	3
11903	41	4,700	2,510	5 50	4
11215	11	4,800	4,730	5 11	4
11831	2	4,800	4,730	4 82	5
11852	12 (subsoil)	4,900	4,400	4 74	5
	Average	4,527	4,185		
11321	127	5,000	5,160	5 79	2
11279	12	5,200	4,300	4 88	5
S6396	41	5,200	4,370	5 16	3
11236	12	5,300	6,450	5 00	4
S6399	41	5,300	3,440	5 45	3
11328	127	5,500	4,300	5 28	3
11851	12	5,950	5,600	4 51	5
	Average .	5,350	4,803		

TABLE 1—Concluded

LABORATORY NUMBER	TYPE NUMBER*	BASES REQUIRED CALCULATED	BASES REQUIRED BY Na₂CO₃ METHOD	pH	THIOCYA- NATE†
		pounds	pounds		
11851	12	6,100	6,020	4 51	5
11853	12 (subsoil)	6,600	6,400	5 17	5
12089	56	7,600	4,300	5 56	3
11801	1	7,800	5,590	4 86	4
11813	1	8,200	5,160	5 00	5
11880	21	8,900	9,460	5 17	3
11837	3	10,800	9,460	4 80	5
11856	12 (subsoil)	11,800	13,200	5 06	5
11854	12 (subsoil)	13,500	16,800	5 13	5
11855	12 (subsoil)	14,600	16,400	5 17	5
	Average	9,590	9,279		

In the foregoing discussion the term "lime requirement" has been used to designate the amount of $CaCO_3$ necessary to raise the degree of saturation with bases to 80 per cent. Other factors which may influence the efficiency of the limestone added in bringing about this degree of saturation must also be considered.

One of these factors is the fineness of the limestone. Coarse particles which remain in the soil for years may serve as alkaline centers, yet neutralize only a small part of the surrounding soil. It may be possible by adding enough of these coarse particles to furnish sufficient alkaline centers to promote sweet clover growth. Pot culture experiments at this station represented by the following data indicate that where limestone which passes through an 8-mesh but over a 10-mesh sieve is used, more than twice as much is required for good sweet clover growth as of fine limestone (through 100 mesh).

RATE PER ACRE 2,000,000 POUNDS SOIL	CONDITION OF SOIL AT END OF 2 YEARS	SWEET CLOVER YIELDS	
		2 years after liming	5 years after liming
		gm	gm
7,000 pound 100-mesh stone	pH 6 63 74 per cent saturated	20 1	17 2
10,000 pound 8–10-mesh stone	pH 5 56 44 per cent saturated	12 1	15 1
2,000 pound 100-mesh stone	pH 5 57 38 per cent saturated	6 6	0
No limestone	pH 4 82 29 per cent saturated	0	0

The soil receiving 2,000 pounds of fine, high-calcium stone grew a small crop of sweet clover the first time only, whereas the soil receiving 10,000 pounds of coarse stone continued to grow sweet clover for more than 5 years but with a lower yield than was obtained on the soil receiving 7,000 pounds of fine material.

It is evident that the coarse particles, besides slowly raising the degree of

saturation, are serving as alkaline centers which are used by the sweet clover. The growth of sweet clover, however, is not satisfactory and a system of liming depending on these large particles alone would require enormous amounts of such limestone on strongly acid soils. Therefore, the effectiveness of the limestone in raising the degree of saturation should be the main consideration in evaluating limestones of varying degrees of fineness. Average limestone screenings, as used in Illinois, were found, when applied to a highly acid gray silt loam soil, to be from 75 to 80 per cent effective over a 5-year period as compared to finely pulverized limestone. The ineffective portion consisted, in the main, of coarse particles recoverable after the 5 years as $CaCO_3$.

Another factor in considering the amount of limestone required to hold good over a period of years is the rate of leaching. Once a high degree of saturation is reached, any excess of fine $CaCO_3$ is soluble in the slightly carbonated rain water and is fairly rapidly taken down below the plowed depth. However, if no excess $CaCO_3$ is added and all the Ca applied is taken up in the replaceable form, the loss is slower compared to the loss incurred in trying to maintain a high degree of saturation with excess limestone present. That the rate of loss of replaceable calcium increases as the degree of saturation increases was indicated in this laboratory by leaching soils of different degrees of saturation with carbonated water. It is considered by the writers to be uneconomical as well as unnecessary to try to maintain a degree of saturation above 80 per cent.

In the pot culture work referred to in the foregoing, an average loss of 23 per cent was observed, part of which may have been due to leaching and part to another factor to be considered next.

Crowther (5) mentions that the change in pH which occurs in the field due to liming is less than that shown in the laboratory. Pierre and Worley (11) in estimating the amount of lime necessary to bring the soil to definite pH values by means of the buffer method and the determination of exchange hydrogen, finds that a factor of 1.5 is necessary to correct for inefficiency in the action of the lime compared to the laboratory methods where the efficiency is measured by adding known amounts of lime to small glasses full of moist soil and allowing them to stand for a relatively short period. MacIntire and Sanders (9) found a loss of lime approximating 16 per cent which could not be accounted for in the replaceable or carbonate forms or in the drainage waters. They think it indicates a combination "with soil components other than the exchange complex."

Whatever the cause of this loss, it must be charged against the efficiency of the limestone in raising the degree of saturation. MacIntire and Sanders' total loss, including the drainage loss, amounted to about 21 per cent of the amount added. This compares favorably with the loss of about 23 per cent of the amount decomposed as found by the writers. Although one figure is based on amounts added and the other on amounts decomposed, they can be compared since in both cases practically all of the limestone was decomposed except in a few instances where large particles were used.

Values have been computed from Lunt's (8) work on total and replaceable calcium in soils from eight Illinois experiment fields, which indicate that this possible "fixation" may be very slight in long-time experiments where large amounts of calcium carbonate have been added. Only one field, Ewing, out of the eight fields studied, shows any appreciable increase in total calcium on the limed plots which is not accounted for in the replaceable or carbonate forms. The limestone additions have in some cases doubled the total calcium content of the soil and the comparison of total amounts in adjacent limed and unlimed plots should be fairly significant, subject only to variation in the total amounts originally present.

In the field plots, however, the total amount of calcium added as limestone through a period of years cannot be accounted for in the surface, a large percentage having been lost from the plowed depth. In these field experiments the limestone was added in excess of the amount required, as high as 20,000 pounds per acre having been added in some cases over a period of years to soils with base-exchange capacities equivalent to 10,000 pounds. The statement made earlier in this paper that loss due to leaching is greater where excess $CaCO_3$ is present and the base-exchange complexes are highly saturated is borne out by these heavy losses on highly limed plots and also by the relatively small losses of replaceable bases which have been suffered by acid check plots over periods up to 25 years.

In using this titration method, the aforementioned factors should be considered and corrections made for them. It has been decided that, for the present, the titration value should be multiplied by 1.67. This gives the limestone screenings used in this state an efficiency of 60 per cent based on 100 per cent $CaCO_3$ equivalent. Twenty per cent of the reduction is assumed to be due to the coarseness factor and 20 per cent is considered a safety factor, which includes any relatively early loss of the available forms by leaching, as well as any possible "fixation." In the case of finely ground limestone (all passing a 35-mesh Tyler sieve), the correction for coarseness is omitted and the efficiency factor is, therefore, 1.25.

THE LABORATORY METHOD

Procedure.—To each of a series of 5-gm. samples of 10-mesh, air-dry soil (not ground) in test tubes add 1 gm. KCl and 25 cc. distilled water. To the first, add standard Na_2CO_3 solution equivalent to 0.0062 gm. $Na_2CO_3 \cdot H_2O$. This is equivalent to 1 ton of $CaCO_3$ to an acre, 2,000,000 pounds of soil. To the others add two, three, and four times this amount, respectively. Half-ton intervals instead of 1-ton may be used if desired. Shake vigorously about 25 times and let settle. Then add three drops of bromthymol blue to each tube and agitate very gently, just enough to mix the indicator with the upper half-inch of solution. The first tube showing a green to greenish blue color is considered as having had sufficient base and the corresponding amount of $CaCO_3$ is taken as the lime requirement. When half-ton intervals are used,

the color in the tube just below the one giving the endpoint will usually be yellow, and the one just above will be blue. A yellow-green color should be taken as the endpoint only in case the tube having the next larger amount of Na_2CO_3 shows a blue. If the colors of two adjacent tubes are yellow and blue, respectively, the endpoint may be taken as half way between these two. By making a preliminary thiocyanate test, or one of the available indicator tests, it is usually possible to reach the endpoint with only three trials, using, for example, 1-, 2-, and 3-ton equivalents for slightly acid soils and 3-, 4-, and 5-ton equivalents for those apparently more acid. In the original trials reported in table 1, an amount of Na_2CO_3 solution equivalent to 430 pounds of $CaCO_3$ an acre, or a multiple, was used in the different trials. This interval, however, is too small to give an endpoint sharp enough for practical use.

Although this method as outlined could be made portable for field use, the possible difficulty of maintaining properly standardized Na_2CO_3 solution and calibrated medicine droppers in lieu of burettes led to the following modification which is designed for use in the field.

THE FIELD METHOD

Materials.—(a) $Na_2CO_3 \cdot H_2O$-KCl tablets. Mono-hydrated sodium carbonate, $Na_2CO_3 \cdot H_2O$, and KCl are ground together and compressed into tablets such that each tablet contains 0.5 gm. KCl and 0.0062 gm. $Na_2CO_3 \cdot H_2O$; (b) bottle of distilled water; (c) bromthymol blue in dropping bottle; (d) mortar and pestle for crushing soil; (e) spatula; (f) special 5-gm. measuring cup (5 cc. capacity); (g) test tubes, 3/4 inches by 6 inch and test tube rack; and (h) graduated cylinder, 25 cc.

Procedure.—Measure three to four 5-gram samples into separate test tubes. The measuring cup is filled and slightly heaped by pouring the crushed, but unground soil, into it with the spatula, avoiding any shaking or jarring of the cup since this would cause packing. When full, strike off level with the edge of the spatula. Except for sands, mucks, and peats, these samples will vary only slightly if the measuring is properly done. Add one tablet to the first tube, two to the second, and so on. The tablets should be crushed in the measuring cup with the end of the spatula handle before adding to the soil in the test tubes, unless they are soft enough to dissolve quickly. Then add 25 cc. of water to each sample, shake until tablets are completely dissolved, let settle, add bromthymol blue, and read the endpoint as in the laboratory method

The tablets, as described in the foregoing, can be made satisfactorily by manufacturing pharmacists. A trial lot made by Parke, Davis and Company were practically constant in neutralizing value. Different batches might vary slightly and, therefore, require standardization.

In the field method, the addition of increasing amounts of Na_2CO_3 for titration is accompanied by increases in the amount of KCl used. This, it has been found, does not appreciably influence the results, as compared to the

laboratory method in which a constant quantity of KCl is used with varying amounts of base.

In case half-ton intervals are desired in the field method, tablets could be made to contain 0.25 gm. KCl and 0.0031 gm. $Na_2CO_3 \cdot H_2O$.

In the foregoing directions, no account has been taken of impurities in the limestone or other factors inimical to its effectiveness. As a matter of fact the tablets now in use at this station have been corrected for these factors, each tablet being equivalent to 1 ton an acre of average commercial limestone screenings as used in Illinois, the amount of $Na_2CO_3 \cdot H_2O$ present in each tablet being only 60 per cent of the theoretical amount. The reason for this reduction in amount has already been discussed.

APPLICATION OF THE METHOD TO A FIELD-TESTING PROGRAM

This quantitative field method is adapted for use in conjunction with the field testing and mapping plan as used in the Illinois soil extension program (7). This plan, which is being used successfully among Illinois farmers, requires the testing of 23 soil samples, uniformly distributed over a 40-acre field, by means of the potassium thiocyanate test. As a result of these tests, the field is mapped into areas of possibly high, medium, low, and no acidity. The interpretation of the three degrees of acidity, in terms of the amounts of limestone needed, is largely a matter of experience. Years of experience on the part of extension workers has resulted in a practical calibration of the test, and a color chart is now being used which has been found to be suitable for the average soil conditions. The deviation from this average condition, however, is likely to be large in certain soil types, and cannot be recognized even by the experienced soil tester.

The carrying out of the following plan enables one to make a definite quantitative interpretation of the thiocyanate test or any indicator test which may be used. All the samples from a given field on the same or related soil types showing the same degree of acidity by the qualitative test are composited. This gives a maximum of three samples from any field to be tested by the titration method, i.e., the "low acidity" samples, the "mediums," and the "highs." Thus the exact meaning of "low," "medium," and "high" acidity as mapped on this particular field is found in terms of pounds of limestone needed per acre. The areas testing non-acid by the thiocyanate test where proper precautions are taken[7] need no limestone and a further quantitative test would be superfluous.

The field method, as described in this paper, is not sufficiently rapid to be used on a large number of samples as is the thiocyanate test. Therefore, it is recommended that the thiocyanate test or similar indicator test be used for

[7] Certain soil types which are acid do not give a red color with the thiocyanate test unless a small amount of finely divided iron is added before shaking the mixture. This deficiency in that form of iron which gives the test seems to be associated with mature soils formed under conditions of good drainage.

the qualitative separation and mapping of the field so that the number of samples to be run by the titration method on a given 40 acres will be reduced to three or less. By taking advantage of both of these methods, a quantitative value is found for a rather large area with a minimum number of samples.

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SOME INFLUENCES OF THE DEVELOPMENT OF HIGHER PLANTS UPON THE MICROÖRGANISMS IN THE SOIL: IV. INFLUENCE OF PROXIMITY TO ROOTS ON ABUNDANCE AND ACTIVITY OF MICROÖRGANISMS[1]

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HISTORICAL

In recent publications (64, 65, 66) it has been shown that plant development exerts pronounced effects upon the microbial population of the soil. The bacteria developing upon albumin agar and the bacteria closely related to *Bacillus radiobacter* were much more numerous in soil about plant roots than in soil free from roots. The activity of the organisms as measured by the rate of formation of carbon dioxide and nitrate was much greater in the soil about roots. Specific differences were noted with different plants, particularly between annuals and biennials, and these differences were correlated with certain characteristics of growth of the plants. Actinomyces and fungi responded less to the plants than did the bacteria. Considered as a whole, however, the increase in these organisms was statistically significant (67). The results obtained during the second year of growth of three of the biennials are presented elsewhere (67). These results indicated that there is no marked decrease in abundance and activity of microörganisms while the plants are in a vigorous vegetative condition. In fact, during the second year, greater numbers of bacteria were observed than at any period of the first year. Evolution of carbon dioxide remained at a high level and nitrification was more active than at any preceding period of observation.

The results obtained by Velich (76), Hoffman (22), Joshi (27), Wilson and Lyon (81), Löhnis (38), and Smith (63) have been discussed in a previous report (64) and it need only be mentioned here that their results indicate that bacteria are more abundant about roots of developing plants. .

Negative results were obtained by Creuzburg (9) who could demonstrate no increase in microörganisms or biological activity about plant roots. Headden (15) concluded that plants even depress microbial activities in soils. This conclusion is merely speculative, in view of the fact that it is based not upon determinations of the abundance of organisms but upon determinations of

amounts of carbon dioxide in soil air. The observations of Vande Velde and Verbelen (75) indicate that, in soil which was not treated previous to planting, the number of bacteria diminished during germination of seeds. The small number of observations made and the limitations of the method used render these results inconclusive.

The results recently reported by Gräf (14) are particularly pertinent in connection with those to be discussed in the following pages. She found much larger numbers of the general bacterial population and also of members of the Radiobacter group in soil about plant roots. Even more interesting is the fact that upon the root surfaces there appeared many times as many organisms as in the soil adjacent to the roots. This study included observations with eight different kinds of plants. She made attempts to determine the capacity of organisms in the soils and root materials to form ammonia from peptone, to fix nitrogen, to oxidize ammoniacal nitrogen, and to assimilate nitrate nitrogen. These results give no indication of the activity of organisms about roots, because the methods used were not suitable for deriving this information. The fact that no pronounced increase was noted in the activity of organisms about roots does not justify the idea that microörganisms are no more active on root surfaces than in soil free from roots.

There is considerable evidence to indicate that carbon dioxide is produced in greater amounts in soils supporting plant growth than in soils devoid of plants (30, 69, 32, 1, 33, 56, 2, 74; see also recent review by Thomas, 70). Furthermore, greater amounts are produced during advanced periods of vegetative growth, during blooming, or fruiting, and there is a pronounced decrease during degeneration of the vegetative portion of the plant. Metzger (44) observed similar changes in the concentration of bicarbonates about roots. Russell and Appleyard (58, 59) obtained less conclusive results but nevertheless believed that the growing crop is a factor affecting formation of carbon dioxide in soil.

Little information is yet available to indicate what portion of the increase in carbon dioxide about roots is the result of microbial activity. It has been shown previously (64, 65, 66, 67, 14) and will be indicated in the following pages that microörganisms are far more numerous about roots and on root surfaces than in soil devoid of roots. Fred and Haas (12) demonstrated that the activity of microörganisms on root surfaces greatly increases the solvent action exerted by roots.

In view of these facts and that the author noted that greater amounts of carbon dioxide were produced by soils obtained from the region of root development, it was concluded that " . . . . at least part of the increase in carbon dioxide in the soil air under plants and increase in bicarbonates about plant roots should be ascribed to microbial action" (66, p. 438). This statement and those accompanying it seem conservative, in view of the fact that no other explanation seems likely to account for the vital activities of the tremendous numbers of microörganisms found about roots. Thomas (70) considers that the total contribution to the carbon dioxide by microörganisms is insignificant compared

with the amount evolved during the growing season by the respiratory activity of the roots.   The author still persists that, notwithstanding the evidence offered by Thomas to the contrary, the information which is available at the present time does not permit the statement in any definite terms of what portion of the carbon dioxide produced about roots is the product of microbial activity and what originates from respiration of the root cells.   The evidence presented by Thomas is that obtained from Barakov (1), Headden (15), and Turpin (74), all of whom made determination of the amounts of carbon dioxide in the soil air.   These measurements of necessity included the sum of the amounts of carbon dioxide originating from all the various sources within the soil and permit no distinction between those amounts arising through microbial action and those coming from respiration of root cells.

There are many factors affecting the concentration of carbon dioxide about plant roots and thus rendering difficult an accurate interpretation of results. The amount of carbon dioxide in soil is no adequate indication of the speed with which it is being formed.   Factors modifying rates of diffusion will create differences as great as factors modifying production (41).   It can be imagined that even under plant cover, in certain instances there may be less carbon dioxide than in fallow soil, as where the plant increases the rate of diffusion by such changes as lowering the water content and making the soil more porous by root penetration.

Measurements of gas formation in solution cultures of plants where microorganisms are not eliminated are open to the criticism that microörganisms in the cultures may be responsible for the formation of certain amounts of the gas.

Neller's results (46) further complicate an interpretation of studies of influences of plant development on production of carbon dioxide in soils, since they suggest that development of plants in soil causes an acceleration in the rate of transformation of the organic soil constituents.

Although there may be justification for the conclusion that root respiration is responsible for a large contribution to the carbon dioxide of soil air, there is fully as much reason for concluding that microörganisms about roots are responsible for a large part of the increase in carbon dioxide which accompanies the growth of higher plants.   In this connection it may be sufficient merely to cite the results obtained by Lundegardh (41, p. 209) which show that as much as 45 per cent of the carbon dioxide formed about roots may arise from microbial action.

In view of the limited information which is now available concerning the importance of microörganisms as agents of formation of carbon dioxide in the zone of root development of higher plants, it is logical to delay speculations concerning the relative quantities of carbon dioxide contributed by microörganisms until more exact information is obtained.

EXPERIMENTAL PROCEDURE

The specific object of the following experiments was to determine what differences exist between the abundance and activity of microörganisms on the imme-

diate surfaces of plant roots, in soil close to the roots, and in soil farther removed from the zone of root development. This followed the anticipation expressed in a preceding publication (65) that, if it is assumed that root products are of major importance in determining the pronounced increases in microörganisms in the soil about roots, greater effects should appear at the root surfaces. These experiments were conducted during the months of June to October, 1929. It is interesting in this connection that the experiments of Gräf (14), which in some respects are similar to those to be reported, appear to have been performed simultaneously.

Two series of plants were used. One series consisted of second year growth of the three biennials, table beets, mangel beets, and sweet clover. Representatives of the same planting had been used during growth the first year for studies already reported (65, 66). In most cases three samples were taken about each plant as follows: (a) At 15 cm. distant from the main root of the beets and 30 cm. laterally from the base of the sweet clover to a depth of 12 to 15 cm.; (b) from the region of development of the roots of the plants to a depth of 12 to 15 cm.; (c) from the immediate root surfaces after all of the soil material which was readily shaken off had been removed. This last sample was obtained by scraping off the thin superficial layer of the root surface with a sterilized steel spatula. It consisted of both soil material clinging to the root surfaces and the outermost portion of the root tissue. These various samples were gathered about the plants at three periods during the summer. At these times the mangel beets and table beets had been planted for 375, 399, and 456 days. The sweet clover plants were 356, 380, and 437 days old at these periods.

The second series of plants consisted of a new planting of bush beans, mangel beets, and field corn. An attempt was made to confine the roots but at the same time create conditions which would permit free movement of materials between the region where the roots were confined and the remaining body of the soil. To accomplish this purpose, the seeds were planted within closely woven wire cylinders. The cylinders were prepared of 30-mesh tinned wire. They were sunk into the ground to the depth of 40 cm., projecting slightly above the surface. The cylinders were either 10, 15, or 20 cm. in diameter. The different sizes were used in order to accommodate the plants as they developed during the season. It was hoped that the wire would confine the roots so that samples of soil taken at various distances from the outside of the cylinders would represent soils occurring at certain regions away from the roots of the plants. By far the greatest portion of the roots was retained by the wire screen. However, the wire corroded somewhat, and many roots penetrated the screening even where it did not corrode. Consequently, the samples which were obtained did not represent soils free from roots in most cases but soils exposed to different degrees of root penetration.

The plants were well isolated from one another so that there was no danger of root systems from one plant penetrating the soil about the region of development of another. The samples were obtained by the following procedure. An

amount of soil was dug out, to the depth of about 17 cm., in the form of a circular trench just outside the region 30 cm. from the position of the wire cylinder. A thin layer of soil was taken from the entire cylindrical exposed surface, which was 30 cm. distant from the wire cylinder and 17 cm. deep. This was thoroughly mixed together and a representative portion was put through a 3-mm. sieve. A sample of about 1,000 gm. of sieved soil was brought to the laboratory for various determinations.

After the first sample was obtained, soil was removed up to the region 15 cm. distant from the wire cylinder. Here a second sample of soil was taken in a manner similar to the aforementioned. Then most of the soil surrounding the cylinder to a depth of 17 cm. was removed, leaving only a layer 3 cm. thick about the cylinder. This layer of soil surrounding the screening was removed, mixed, and sieved. This was sample 3.

The cylinder of soil was next removed, and the soil contained in the cylinder to a depth of 17 cm. was separated from the roots. This was sample 4. The roots were brought to the laboratory where they were scraped. The soil particles which were thus removed from the root surfaces, together with the superficial root tissues and rootlets were gathered. This was sample 5. Such samples of material were gathered about these plants at two periods during the summer, first, after the seed had been planted 66 days, and second, 113 days subsequent to planting.

All of the samples were examined for: (a) abundance of bacteria developing upon albumin agar, (b) abundance of *Bacillus radiobacter* and related forms developing on glycerol-nitrate-soil-extract agar, (c) abundance of total and mucoid colonies appearing on nitrogen-free mannite agar, (d) abundance of actinomyces developing on albumin agar, and (e) abundance of fungi growing on an acid medium. The procedures used were those followed previously (64, 65).

Determinations were also conducted on the soil samples for the evolution of carbon dioxide, the amounts of nitrate nitrogen produced from the soil nitrogen in 30 days, and the amounts of nitrate nitrogen formed in 6 days in the presence of added ammonium sulfate and calcium carbonate. These determinations were outlined previously (66). All the soils were brought to 25 per cent moisture at the beginning of these determinations.

The type of material represented in the samples obtained from the root surfaces was not adapted to these measurements of biological activity, being both limited in quantity and containing large amounts of root tissue.

The soil with which these experiments were conducted was a Sassafras loam. In reaction it varied between pH 6.0 and 7.2 during the season; most frequently the reaction was close to neutrality. The plants all made excellent growth.

The microörganisms would become very incompletely dispersed in the diluting liquid from the sample obtained from the root surfaces by simple shaking in water preliminary to plating. Consequently, the material was triturated in a mortar, aseptic precautions being observed. The weighed sample was added

to a sterile mortar.　Sufficient sterile water was added to make a thin paste and the material was triturated with a sterile pestle until a smooth, finely ground

TABLE 1

Moisture contents of soil and plant materials

Second year of biennials

PLANT	REGION OF SAMPLING	AGE OF PLANT AT PERIOD OF SAMPLING		
		375 days	399 days	456 days
		per cent	*per cent*	*per cent*
Table beet....	15 cm. from main root	19 8	23 1	18 8
Table beet.... .	Close to main root	20 2	21 9	17 2
Table beet.	Superficial layer of the root	...	52 0	22 8
Mangel beet..	15 cm. from main root	20.1	21.5	19 5
Mangel beet...... .	Close to main root	18 3	22 5	18 1
Mangel beet.....	Superficial layer of the root	..	51 6	37.1
Sweet clover*..... .	30 cm. from main roots	16 3	20 5	16 4
Sweet clover*.......	Close to main roots	11 8	19 5	17 0
Sweet clover*... ..	Superficial layer of the roots	. ..	32 7	24 1

First year of a planting

PLANT	REGION OF SAMPLING	AGE OF PLANT AT PERIOD OF SAMPLING	
		66 days	113 days
		per cent	*per cent*
Bean	30 cm. from wire	...	20.3
Bean.......	15 cm. from wire ′	18.5	20.4
Bean	Close to wire	17 5	20 6
Bean........ .. .	Close to main roots	17 6	20 0
Bean.....	Superficial layer of the roots	...	16.8
Mangel beet..... .	30 cm from wire	....	20.6
Mangel beet... .	15 cm. from wire	17.5	20.0
Mangel beet...	Close to wire	16 4	18 3
Mangel beet....	Close to main root	15 6	16 3
Mangel beet.......	Superficial layer of the root	20.2	23.5
Corn	30 cm. from wire	....	18.8
Corn...........	15 cm. from wire	13.9	17.7
Corn...................... .	Close to wire	14.2	15.8
Corn........	Close to main roots	13 2	15.8
Corn......................	Superficial layer of the roots	27.5	19.2

* For sweet clover the sampling periods are 356, 380, and 437 days.

substance was obtained.　This was transferred to a sterile flask with sufficient sterile water to make a known initial dilution.　Although not all of the soil

samples were treated in this manner, it is believed that this did not appreciably affect the general nature of the results. This conclusion seems justified from the results obtained where the soils were triturated the same as the root samples. All of the samples taken the second period about the plants 113 days old were triturated. These results are in general agreement with the others.

The results on abundance of organisms are reported on the basis of numbers per gram of soil material as taken from the field. The moisture contents of these samples are presented in table 1.

<div align="center">EXPERIMENTAL RESULTS</div>

Abundance of Microörganisms

The results of determinations of the abundance of bacteria are shown in table 2. It is quite apparent that the plants exert pronounced effects upon the development of bacteria. The most striking effect appears at the immediate root surfaces where the organisms are many times as abundant as in the soil surrounding the roots. The least effect was observed under beans at the 113-day period, where 3.6 times as many bacteria were found on the roots as in the soil close to the roots. The greatest effect was observed under sweet clover at the 437-day period. Here 120 times as many bacteria were found on the roots as in the soil about the roots. It is not surprising that large numbers of bacteria were found on or within the roots of legumes because of the well-established relationship of specific bacteria with these plants. That there is similar extensive development of bacteria on roots of all of the plants is of particular interest.

Averages of the effects of all the plants show that 24.8 times as many bacteria were found on the plant roots as in the soil close to the roots. Exclusive of the legumes, there were 12.1 times as many bacteria. The results of the legumes alone show 50.3 times as many bacteria on the root surfaces. Except for the abundance of bacteria on or within the root surfaces, there is nothing distinctive about the effects of the legumes. In general there were as many organisms in the soils about the roots of non-legumes as the legumes.

It is also apparent from the results in table 2 that bacteria are less abundant in soils at considerable distances from the region of origin of the roots. Under the second year growth of biennials, with only one exception, the soils about the roots show considerably greater numbers of bacteria than the soils 15 cm. from the main mass of roots. These effects appear fully as striking under the plants 113 days old, because more samples were taken at various regions from the location of the principal roots. In these cases, there is a progressive increase in the abundance of bacteria the nearer to the plants the soils were obtained. These specific effects of the different plants are more readily understood when it is explained that no root development was apparent at this period 30 cm. from the wire under either beans or mangel beets, but was noted at this distance under corn. There were a few roots in the soil supporting beans and mangel beets at a distance of 15 cm. from the wire.

The younger bean and mangel beet plants (66 days) showed little effect of root development on the bacteria except on the root surfaces. By reason of its more extensive root development at this time, corn produced more apparent

TABLE 2

Influence of distance from roots on total bacteria in soil

(Numbers at dilution of 1/1,000,000)

Second year of biennials

PLANT	REGION OF SAMPLING	AGE OF PLANT		
		375 days	399 days	456 days
Table beet	15 cm from main root	18 6	23 2	22 6
Table beet	Close to main root	49 4	38 2	49 0
Table beet	Superficial layer of the root	458 4	737 4	485 0
Mangel beet	15 cm from main root	22 8	15 6	16 2
Mangel beet	Close to main root	18 8	61 6	44 4
Mangel beet	Superficial layer of the root	604 6	462 8	583 0
Sweet clover*	30 cm from main roots	18 0	31 2	16 2
Sweet clover*	Close to main roots	35 0	56 2	28 8
Sweet clover*	Superficial layer of the roots	887 4	3 637 8	3 470 0

First year of a planting

PLANT	REGION OF SAMPLING	AGE OF PLANT	
		66 days	113 days
Bean	30 cm from wire		18 6
Bean	15 cm from wire	17 4	32 8
Bean	Close to wire	20 8	36 2
Bean	Close to main roots	17 6	55 4
Bean	Superficial layer of the roots	662 4	199 4
Mangel beet	30 cm from wire		18 6
Mangel beet	15 cm from wire	16 2	27 0
Mangel beet	Close to wire	14 8	33 4
Mangel beet	Close to main root	20 5	57 4
Mangel beet	Superficial layer of the root	92 4	427 4
Corn	30 cm from wire		22 8
Corn	15 cm from wire	14 2	26 2
Corn	Close to wire	25 4	44 8
Corn	Close to main roots	122 0	93 2
Corn	Superficial layer of the roots	1,315 6	653 4

* For sweet clover the sampling periods are 356, 380, and 437 days

effects. Roots of corn penetrated to the region of 15 cm. from the wire at this period. Roots of beans and mangel beets scarcely penetrated the wire screening at this time.

From these data it is quite reasonable to suppose that the organisms are most affected where there is abundant development of roots; where root development is relatively scant, few bacteria appear.

Similar but more pronounced influences of plants were observed upon the abundance of *B. radiobacter* and related forms. The results are shown in table 3. As in the case of the general bacterial population, the organisms were found in far greater abundance on the root surfaces than in the soils close to the roots. Considering all of the plants together, there were 45.3 times as many bacteria on root surfaces as in soils close to the roots. Exclusive of legumes, these bacteria were 11.2 times as numerous on the roots. With the legumes alone, 113.3 times as many of these bacteria were found on the roots as in the soil surrounding the roots. It is natural to expect that these organisms would be proportionally greater in or on roots of legumes than on roots of non-legumes since *Bacillus radicicola* is included in the group and undoubtedly made up a considerable portion of the bacteria developing from samples taken from legume roots. Nevertheless, it is of particular interest that very large numbers of organisms of the Radiobacter group occur on the surfaces of roots of non-leguminous plants. Their significance still remains undetermined but the fact that they were found, indicates that they were able to obtain the necessary food materials in the environment about the roots.

There is a pronounced decrease in abundance of these bacteria the greater the distance the soils occur from the region of most extensive root development. This decrease is considerably more apparent than with the general bacterial population. In the case of the soils under the second year of biennials there were 2.1 times as many total bacteria in soils about the roots as in soils 15 or 30 cm. from the main roots. However, there were 4.5 times as many bacteria of the Radiobacter group. In the case of the soils supporting the first year growth plants, 3.1 times as many total bacteria were found close to roots as 15 cm. distant. In the same soils, 29.3 times as many bacteria of the Radiobacter group were found close to the main roots. These results are in agreement with previous observations which indicated that proportionally small numbers of organisms of the Radiobacter group occur in soils free from plant development (63, 65, 14). It is logical to conclude that organisms of the Radiobacter group are affected by plant development proportionally more than is the bacterial population as a whole.

Organisms developing upon mannite agar showed response similar to that of the general bacterial population (table 4). The bacteria were far more numerous on the root surfaces than in soil surrounding the roots. These organisms were much less abundant in soils distant from the central root systems and, as a rule, the greater the distance from the regions of most extensive root development, the lower the numbers of bacterial inhabitants.

Table 5 presents results of determinations of the bacteria which produce mucoid colonies on mannite agar. These bacteria are undoubtedly much the same as those referred to previously as members of the Radiobacter group.

The numbers are somewhat higher, possibly because, on this medium, certain bacteria were able to develop which were inhibited by the crystal violet in the

TABLE 3

Influence of distance from roots on Radiobacter developing on glycerine-nitrate agar

(Numbers at dilution of 1/10,000)

Second year of biennials

PLANT	REGION OF SAMPLING	AGE OF PLANT		
		375 days	399 days	456 days
Table beet	15 cm from main root	16 0	14 4	7 0
Table beet	Close to main root	21 4	16 2	14 0
Table beet	Superficial layer of the root	113 6	110 4	92 0
Mangel beet	15 cm from main root	1 4	4 2	4 6
Mangel beet	Close to main root	15 6	12 8	12 0
Mangel beet	Superficial layer of the root	78 4	472 0	72 0
Sweet clover*	30 cm from main roots	2 4	5 2	4 4
Sweet clover*	Close to main roots	14 4	43 2	23 2
Sweet clover*	Superficial layer of the roots	610 0	8,540 0	9,760 0

First year of a planting

PLANT	REGION OF SAMPLING	AGE OF PLANT	
		66 days	113 days
Bean	30 cm from wire		5 2
Bean	15 cm from wire	0 8	3 2
Bean	Close to wire	4 6	8 0
Bean	Close to main roots	11 2	19 6
Bean	Superficial layer of the roots	844 8	164 0
Mangel beet	30 cm from wire		6 6
Mangel beet	15 cm from wire	1 4	11 4
Mangel beet	Close to wire	5 8	13 8
Mangel beet	Close to main root	7 4	63 6
Mangel beet	Superficial layer of the root	202 0	246 0
Corn	30 cm from wire	.	2 4
Corn	15 cm from wire	2 4	4 6
Corn	Close to wire	17 6	10 8
Corn	Close to main roots	322 0	47 8
Corn	Superficial layer of the roots	1,412 0	476 0

* For sweet clover the sampling periods are 356, 380, and 437 days

other medium. The general conclusions from the results of these determinations are practically identical with those reported for the Radiobacter group. Doubtless the bacteria were very much affected by plant development and were

particularly favored by conditions on the root surfaces where extensive multi-plication of the bacteria took place

TABLE 4

Abundance of colonies of bacteria on mannite agar developing from soils obtained at different distances from the main roots of plants

(Numbers at dilution of 1/1,000,000)

Second year of biennials

PLANT	REGION OF SAMPLING	AGE OF PLANT		
		375 days	399 days	456 days
Table beet	15 cm from main root	18 0	24 0	8 8
Table beet	Close to main root	44 0	32 6	20 8
Table beet	Superficial layer of the root	358 4	560 0	201 2
Mangel beet	15 cm from main root	19 3	21 0	13 8
Mangel beet	Close to main root	31 2	44 0	19 6
Mangel beet	Superficial layer of the root	556 2	396 0	228 2
Sweet clover*	30 cm from main roots	22 8	16 8	11 8
Sweet clover*	Close to main roots	29 0	46 0	16 6
Sweet clover*	Superficial layer of the roots	935 4	3,028 0	2,864 0

First year of a planting

PLANT	REGION OF SAMPLING	AGE OF PLANT	
		66 days	113 days
Bean	30 cm from wire		7 8
Bean	15 cm from wire	4 8	13 2
Bean	Close to wire	4 2	11 6
Bean	Close to main roots	9 6	25 6
Bean	Superficial layer of the roots	351 8	98 4
Mangel beet	30 cm from wire		8 4
Mangel beet	15 cm from wire	5 8	11 4
Mangel beet	Close to wire	10 4	20 4
Mangel beet	Close to main root	11 6	41 4
Mangel beet	Superficial layer of the root	54 2	130 8
Corn	30 cm from wire		13 2
Corn	15 cm from wire	7 6	15 8
Corn	Close to wire	14 0	27 4
Corn	Close to main roots	91 4	55 6
Corn	Superficial layer of the roots	523 2	300 0

* For sweet clover the sampling periods are 356, 380, and 437 days

As seen from table 6, the actinomyces represent a group of microorganisms which are affected to a very slight degree by plant development. There is

little suggestion of similarity between these results and those previously re-ported for the bacteria. The data for the first year plants show no influence of

TABLE 5

Abundance of mucoid colonies of bacteria on mannite agar developing from soils obtained at different distances from the main roots of plants

(Numbers at dilution of 1/100,000)

Second year of biennials

PLANT	REGION OF SAMPLING	AGE OF PLANT		
		375 days	399 days	456 days
Table beet .	15 cm from main root	10 4	8 2	2 6
Table beet . .	Close to main root	11 2	12 2	4 6
Table beet	Superficial layer of the root	416 0	472 0	214 0
Mangel beet .	15 cm from main root	4 2	5 6	2 0
Mangel beet .	Close to main root	10 8	24 0	6 0
Mangel beet .	Superficial layer of the root	594 0	316 0	232 0
Sweet clover*. .	30 cm. from main roots	4 4	6 6	2.4
Sweet clover*.	Close to main roots	19 8	34 8	8 8
Sweet clover*. .	Superficial layer of the roots	1,680 0	11,320 0	4,040 0

First year of a planting

PLANT	REGION OF SAMPLING	AGE OF PLANT	
		66 days	113 days
Bean	30 cm from wire	.	2 6
Bean..	15 cm. from wire	1 2	4 2
Bean	Close to wire	1 2	3.8
Bean....	Close to main roots	5 6	17.4
Bean....	Superficial layer of the roots	209 2	89.2
Mangel beet	30 cm from wire	.....	3.0
Mangel beet	15 cm. from wire	1.2	5 4
Mangel beet......... .	Close to wire	2 0	8 8
Mangel beet . . .	Close to main root	2 7	13.2
Mangel beet .	Superficial layer of the root	80 4	83.0
Corn.	30 cm from wire	.....	2 4
Corn..	15 cm. from wire	3.2	6.0
Corn	Close to wire	9 5	18 8
Corn.	Close to main roots	58 6	24 6
Corn.	Superficial layer of the roots	858 0	448 0

* For sweet clover the sampling periods are 356, 380, and 437 days.

root development on the actinomyces which can be considered to be significant. The older plants show some limited effects upon the organisms. Averaging the effects of the second year growth of the biennials, it is noted that there were

3 9 times as many actinomyces found upon the immediate root surfaces as in the soil close to the roots   The differences in abundance of actinomyces on

TABLE 6

Influence of distance from roots on abundance of actinomyces in soil

(Numbers at dilution of 1/1,000,000)

Second year of biennials

PLANT	REGION OF SAMPLING	AGE OF PLANT		
		375 days	399 days	456 days
Table beet	15 cm from main root	5 8	6 6	6 8
Table beet	Close to main root	5 0	6 4	5 8
Table beet	Superficial layer of the root	16 0	18 6	21 0
Mangel beet	15 cm from main root	4 8	8 2	8 8
Mangel beet	Close to main root	3 2	8 2	8 0
Mangel beet	Superficial layer of the root	16 2	21 2	29 0
Sweet clover*	30 cm from main roots	6 2	4 8	5 8
Sweet clover*	Close to main roots	7 0	3 8	4 4
Sweet clover*	Superficial layer of the roots	2 8	10 2	50 0

First year of a planting

PLANT	REGION OF SAMPLING	AGE OF PLANT	
		66 days	113 days
Bean	30 cm from wire		7 6
Bean	15 cm from wire	4 2	10 0
Bean	Close to wire	3 0	8 0
Bean	Close to main roots	5 8	6 2
Bean	Superficial layer of the roots	10 6	12 6
Mangel beet	30 cm from wire		10 0
Mangel beet	15 cm from wire	4 4	11 4
Mangel beet	Close to wire	3 4	10 4
Mangel beet	Close to main root	3 3	6 8
Mangel beet	Superficial layer of the root	2 8	10 6
Corn	30 cm from wire		8 4
Corn	15 cm from wire	5 2	11 8
Corn	Close to wire	6 0	8 8
Corn	Close to main roots	1 0	10 2
Corn	Superficial layer of the roots	2 4	8 6

* For sweet clover the sampling periods are 356, 380, and 437 days

root surfaces and in soils about roots of these biennials are significant statistically.

It appears from these observations that actinomyces may be affected by

development of plant roots, but that the effects are readily detected only with plants in advanced stages of development and even these plants affect the

TABLE 7

Influence of distance from roots on abundance of fungi in soil

(Numbers at dilution of 1/10,000)

Second year of biennials

PLANT	REGION OF SAMPLING	AGE OF PLANT		
		375 days	399 days	456 days
Table beet	15 cm from main root	21 0	25 0	23 0
Table beet	Close to main root	18 8	30 0	26 8
Table beet	Superficial layer of the root	66 0	46 8	64 2
Mangel beet	15 cm from main root	14 0	23 0	17 2
Mangel beet	Close to main root	24 4	30 6	20 0
Mangel beet	Superficial layer of the root	74 2	114 0	47 2
Sweet clover*	30 cm from main roots	23 8	18 2	21 6
Sweet clover*	Close to main roots	17 6	26 0	20 0
Sweet clover*	Superficial layer of the roots	40 4	82 0	212 0

First year of a planting

PLANT	REGION OF SAMPLING	AGE OF PLANT	
		66 days	113 days
Bean	30 cm from wire		24 6
Bean	15 cm from wire	16 6	21 6
Bean	Close to wire	21 2	20 0
Bean	Close to main roots	25 8	19 2
Bean	Superficial layer of the roots	40 9	55 2
Mangel beet	30 cm from wire		25 8
Mangel beet	15 cm from wire	22 8	25 0
Mangel beet	Close to wire	28 2	25 8
Mangel beet	Close to main root	24 0	30 0
Mangel beet	Superficial layer of the root	55 0	156 0
Corn	30 cm from wire		29 6
Corn	15 cm from wire	17 2	23 2
Corn	Close to wire	20 8	29 6
Corn	Close to main roots	25 6	49 6
Corn	Superficial layer of the roots	224 0	278 0

* For sweet clover the sampling periods are 356, 380, and 437 days

actinomyces far less than the soil bacteria. The organisms are more numerous only on the root surfaces and do not show the influences of plant development at

appreciable distances from the region of extensive root development. Previous observations (65, 67) are in general agreement with these results.

Fungi were affected somewhat more than the actinomyces by plant development. In all cases reported in table 7, greater numbers of fungi developed from the materials taken from the root surfaces than from the soils obtained from the regions close to the roots. Averaging the effects of all the plants, it is noted that 3.9 times as many fungus colonies developed from the root surface samples. Although there seems to be a general indication that greater fungus development occurs in soil close to the region of principal root growth than in soil at distances from this region, the results are not statistically significant. Conclusions based upon plate counts of fungi are seldom completely satisfactory, since the amount of material which may be responsible for a fungus colony on a plate is so uncertain. It seems likely, however, that the results are sufficiently consistent to indicate that root development favors the growth of fungi although the effects of the fungi may not be pronounced in regions of the soil not penetrated by large numbers of roots.

Activities of microörganisms

The results concerning formation of carbon dioxide are shown in table 8. In general, they lead to the same conclusion as those recorded previously (66), namely, that root development favors formation of carbon dioxide of microbial origin. In every observation greater amounts of carbon dioxide were produced by soils obtained from the regions of principal root development than from soils obtained at any distances farther removed from the plants. The differences are greater with soils which supported growth of the older plants. This might be anticipated in view of the fact that greater amounts of root residues would be expected to be supplied to the soil organisms from such plants.

It is also apparent that, in soils supporting first year growth of plants, almost without exception, the greater the distance from the region of main root development, the smaller the amount of carbon dioxide produced.

It is unfortunate that no measurements could be made of the amounts of carbon dioxide produced by microörganisms upon the immediate root surfaces. It is in this region that one would anticipate that the greatest microbial activity would take place, since the organisms were many times more numerous than in the soil surrounding the roots. It seems logical to conclude that, since the activity of microörganisms, as indicated by evolution of carbon dioxide, correlates so well with the distribution of bacteria in the soils, carbon dioxide would have been produced in greater amounts by microbes per unit of soil material on root surfaces than from any of the other soil materials investigated and reported in table 8.

There is no doubt that the data do not indicate how much carbon dioxide was produced by the same soils previous to their removal from the field for laboratory study. Judging from the large amount of information available concerning the influence of disturbance of soil on biological activities (79, chapters 28

and 29), there is reason to believe that the carbon dioxide produced by the soils is greater than would have been produced if the soils had remained undisturbed   It seems unnecessary to suppose that this greatly decreases the value of the data.   It is reasonable to conclude that a similar proportionality exists between the amounts of carbon dioxide produced by the various soil samples pre-

TABLE 8

Carbon dioxide evolved from soils obtained at different distances from plant roots

Second year of biennials

PLANT	REGION OF SAMPLING	AGE OF PLANT		
		375 days	399 days	456 days
		mgm	*mgm*	*mgm*
Table beet	15 cm from main root	15 38	21 95	18 38
Table beet	Close to main root	22 55	26 98	22 30
Mangel beet	15 cm from main root	13 25	15 40	14 08
Mangel beet	Close to main root	20 80	25 33	20 95
Sweet clover*	30 cm from main roots	17 85	16 85	12 65
Sweet clover*	Close to main roots	26 80	24 13	18 03

First year of a planting

PLANT	REGION OF SAMPLING	AGE OF PLANT	
		66 days	113 days
		mgm	*mgm*
Bean	30 cm from wire		9 7
Bean	15 cm from wire	11 5	12 0
Bean •	Close to wire	11 3	12 0
Bean	Close to main roots	12 5	15 1
Mangel beet	30 cm from wire		11 2
Mangel beet	15 cm from wire	9 4	13 6
Mangel beet	Close to wire	10 7	14 9
Mangel beet	Close to main root	12 0	18 2
Corn	30 cm from wire		10 3
Corn	15 cm from wire	14 0	15 2
Corn	Close to wire	14 5	15 2
Corn	Close to main roots	20 5	25 0

* For sweet clover the sampling periods are 356, 380, and 437 days

vious to removal from the field and the amounts found during laboratory incubation.   In other words, those soils showing low evolution of carbon dioxide in the laboratory may be considered to have produced small amounts of carbon dioxide in the field; the soils forming larger amounts of carbon dioxide under laboratory conditions probably evolved proportionally large amounts of

carbon dioxide in the experimental plots. Justification for this conclusion is found in the data previously discussed concerning the abundance of micro-organisms in the various samples of soil materials. Those samples which contained a great abundance of microorganisms at the time they were obtained

TABLE 9

Nitrate formation from soil nitrogen during 30 days of laboratory incubation of soils obtained from different distances from plant roots

Second year of biennials

PLANT	REGION OF SAMPLING	AGE OF PLANT		
		375 days	399 days	456 days
		mgm	*mgm*	*mgm*
Table beet	15 cm from main root	1 81	2 20	1 56
Table beet	Close to main root	2 82	2 66	2 12
Mangel beet	15 cm from main root	1 61	1 77	1 42
Mangel beet	Close to main root	2 96	2 00	2 60
Sweet clover*	30 cm from main roots	1 69	1 98	1 52
Sweet clover*	Close to main roots	4 52	3 22	2 49

First year of a planting

PLANT	REGION OF SAMPLING	AGE OF PLANT	
		66 days	113 days
		mgm	*mgm*
Bean	30 cm from wire		0 87
Bean	15 cm from wire	1 41	0 88
Bean	Close to wire	1 36	1 19
Bean	Close to main roots	1 27	1 25
ngel beet	30 cm from wire		1 47
ngel beet	15 cm from wire	0 97	1 46
ngel beet	Close to wire	1 05	1 76
ngel beet	Close to main root	1 77	1 83
Corn	30 cm. from wire		0 76
Corn	15 cm from wire	1 16	1 26
Corn .	Close to wire	1 05	1 52
Corn	Close to main roots	2 07	1 95

* For sweet clover the sampling periods are 356, 380, and 437 days

from the field gave evidence of proportionally high carbon dioxide production upon being incubated in the laboratory. The samples having small numbers of microorganisms showed low biological activity as measured by the formation of carbon dioxide.

Further evidences of increased biological activity in soils about roots is pre-

sented in table 9.   These data indicate that the organic nitrogen of soils obtained from regions of extensive root development is more susceptible to rapid change to nitrate-nitrogen than the organic nitrogen contained in soils which support less root development   Almost without exception, the soils obtained

TABLE 10

Nitrate accumulation in 6 days from the oxidation of ammonia added as ammonium sulfate to soils obtained at different distances from plant roots

Second year of biennials

PLANT	REGION OF SAMPLING	AGE OF PLANT		
		375 days	399 days	456 days
		mgm	*mgm*	*mgm*
Table beet	15 cm from main root	6 00	6 41	8 48
Table beet	Close to main root	8 20	7 70	9 36
Mangel beet	15 cm from main root	5 83	6 00	7 68
Mangel beet	Close to main root	7 40	8 16	9 58
Sweet clover*	30 cm from main roots	7 00	5 50	7 51
Sweet clover*	Close to main roots	7 30	8 43	9 47

First year of a planting

PLANT	REGION OF SAMPLING	AGE OF PLANT	
		66 days	113 days
		mgm	*mgm*
Bean .	30 cm from wire		7 03
Bean	15 cm from wire	7 34	7 59
Bean	Close to wire	8 05	7 59
Bean	Close to main roots	8 55	7 63
Mangel beet	30 cm from wire		8 65
Mangel beet	15 cm from wire	7 16	7 69
Mangel beet	Close to wire	7 27	7 65
Mangel beet	Close to main root	6 73	8 76
Corn	30 cm from wire		8 11
Corn	15 cm from wire	9 30	8 57
Corn	Close to wire	9 43	10 77
Corn	Close to main roots	9 80	9 89

* For sweet clover the sampling periods are 356, 380, and 437 days

from regions of greatest abundance of roots showed most nitrate accumulation With the young plants (66 days), the favorable effects are not as consistent as they were with plants in more advanced development

It is quite possible that similar rapid transformation of the nitrogenous substances occurs in the soils under field conditions.   At least, it is logical to sup-

pose that the soil constituents required by nitrifying bacteria are present in proportionally great abundance in soils close to roots.

The data in table 10 suggests that the nitrifying population in the soil about the roots is able to transform ammonium nitrogen to nitrate-nitrogen somewhat more rapidly than the population of other soils. As with some of the other evidences of influences of plants on soil organisms, the older plants brought about the most pronounced effects. In every instance under second year growth of biennials, greater nitrification was observed in soils close to the main root systems. The results were not quite as consistent under the younger plants, but in general showed the greatest effects of the plants in the regions of most extensive root development. It may be concluded that either the nitrifying organisms in these soils are more efficient transformers of ammoniacal nitrogen or that these soils support a greater abundance of the bacteria.

DISCUSSION

⎣A statement that roots of higher plants and cells of microörganisms exist together in soils would probably remain unchallenged, but various speculations as to the intimacy of relationships between soil microörganisms and root systems might be ventured. With information based upon the results just presented, upon those reported in the preceding papers of the series, and upon the related information gathered together in the following pages, conclusions will be advanced concerning these relationships.

In the body of the soil through which plant roots develop, there is a host of microörganisms having the capacity of attacking and transforming a great variety of organic materials. The region between the interior of root tissues and the soil appreciably removed from root surfaces is of particular interest as regards the activity of microörganisms. In this zone there appear marked changes in the organisms, these changes being initiated by the plants and undoubtedly affecting plant development. The term *rhizosphere* was applied by Hiltner to this portion of the soil where the microbial population is subjected to the effects of plant roots. It was Hiltner's idea that the bacteria develop as a result of root excretions.⎦ The formations consisting of bacteria on the root surfaces and within the roots were called *bacteriorrhiza* (17).⎣ It was considered that each plant favors the development of those bacteria which favor its nutrition. Accordingly, not only each plant group, but each plant, has different root excretions creating a reciprocal relationship between the plant and the bacteria. With changing plant nutrition there is an alteration in the relationship of the rhizosphere flora.

Theories concerning organisms in the rhizosphere have frequently centered about the idea that the relationships of microörganisms to plant roots are beneficial and that the bacteria are specific in their action. Caron (4) believed that, by inoculating soils with bacteria obtained from soils supporting good plant development, he could obtain increased growth of plants. By the use of bacteria isolated from the rhizosphere of beets, for the inoculation of soil,

Hiltner (20) claimed to have obtained increased plant growth. The inoculation of soil with Azotobacter has also been assumed to improve microbial conditions in the rhizosphere (28). Joshi (27) reported that he obtained increased amounts of nitrogen in non-legumes following inoculation of soil with nitrogen-fixing bacteria. Kostytchew (31) observed more favorable development of tobacco in soils receiving additions of cultures of Azotobacter. Truffaut and Bezssonoff (72) claim to have been able to grow corn to maturity with nitrogen-fixing bacteria furnishing the entire supply of nitrogen to the plants. The removal of soluble nitrogenous materials from soil at certain stages of plant development may partly explain the increase in nitrogen-fixing bacteria which has sometimes been observed.

Hiltner (17) conceived the idea that the bacteriorrhiza formations prevent penetration of roots by injurious organisms. More recently, E. Hiltner (16) extended the idea, ascribing to the bacteria the rôle of acting as a filter, protecting the plants against the absorption of injurious minerals.

Since mustard and certain other cruciferous plants are able to develop well in soils apparently deficient in nitrogen, it has been considered that these plants create conditions particularly favorable for the development of Azotobacter in the rhizosphere and that the plants are able to benefit from nitrogen which is fixed by these bacteria (18, 19, 21, 5, 6, 7, 78, 54, 37, p. 20). However, it is not a property specific to cruciferous plants to favor development of Azotobacter. Poschenrieder found Azotobacter generally distributed on roots of a great variety of plants (55).

Stoklasa (68) found that the soil about roots supports an extremely varied assortment of microörganisms, including spore-formers and non-spore-formers, Azotobacter, ammonifiers, denitrifiers, yeasts, and molds. It was believed that the mucilaginous material produced by many of the organisms exerts colloidal effects quite favorable to plant development. Gottheil (13) found many organisms about roots and those he found appeared to be very generally distributed. These observations led him to conclude that it is unlikely that the bacteria play specific rôles about particular plants. Truffaut and Vladykov (73) found a great variety of microörganisms about roots of wheat. Löhnis (37) was of the opinion that differences in the types of organisms existing on the roots of different plants were not as great as some investigators had concluded. Even the differences in types of organisms on roots of cultivated and wild plants did not appear to be pronounced. [Lundegardh considered that the influences of microörganisms upon formation of carbon dioxide about roots could be best explained by assuming that roots are surrounded by a film of soil bacteria which are actively respiring (41).]

Although incomplete, this review indicates that certain particularly important rôles have been ascribed to the organisms occurring about roots of plants. Even with our present limited knowledge of the facts, some of the opinions appear to be highly speculative. Although it is reasonable to suppose that the microbial populations about roots of all plants are not qualitatively alike,

there is no justification for assuming that plants favor the development of only those organisms that are beneficial.

The surprisingly extensive development of bacteria on root surfaces leaves little room for doubt as to whether or not roots supply food material for the development of bacteria even when the roots are apparently healthy. There is considerable direct evidence and other indirect evidence concerning the food substances. From the fact that associative growth of legumes and non-legumes is frequently advantageous to the non-legumes, Lipman concluded that nitrogenous materials are obtained from the legumes either as a result of decay of roots or the elimination of soluble materials from the roots into the surrounding soil (35, 36). Mazé (43) noted the presence of organic substances in nutrient solutions supporting growth of plants. Under more carefully controlled conditions, such elimination of organic materials was found by Lyon and Wilson (42). ⟮Minima (45) presented evidence which led to the conclusion that organic acids are excreted from roots in considerable amounts during periods of advanced vegetative development. It was further noted by Sabinin and Minina (61) that the plants which assimilate phosphorus from tri-calcium phosphate excrete more organic acids than the plants which utilize less of this phosphate.⟯ Demidenko (10) found considerable organic substances excreted into culture solutions by tobacco and corn. It is of particular interest that he observed that the organic compounds are given off in large quantities up to the time when the assimilation processes reach a maximum. ⟮Microbial activity also follows a similar course of change, being greatest at periods of advanced vegetative development or fruiting (65, 66, 67).⟯

Legumes are believed to favor development of symbiotic nitrogen-fixing bacteria about their roots previous to root penetration, through the excretion of amounts of certain organic substances from the roots. Slimy material is frequently excreted about root hairs of many plants other than legumes (80).

In addition to materials excreted by plant roots, certain root parts are regularly sloughed off (80). These include root caps, root hairs, and epidermal cells separated from the roots with advance in age. Information concerning differences in the substances coming from roots of different plants during various periods of growth is not yet available. No doubt it will be shown that the differences are sufficient to affect various elements of the soil population in different ways.

Even greater portions of the roots may be appropriated by soil microörganisms during plant development. Many of the roots which initiate growth, die before the plant is mature, due to loss of vigor brought about by any of a number of soil conditions (80). Decrease in moisture supply may result in the death of roots near the surface; a rise in the water table may cause the death of deep roots. The subterranean portion of the plant continually undergoes changes; the root system extends, loses certain portions, and rebuilds lost parts upon modifications in conditions of temperature, moisture, reaction, composition and movements of gases in the soil (3). The soil conditions are par-

ticularly important as affecting activity of plant pathogens (23, 24, 11, 25, 26, 79, chap. 30). In some cases the environment modifies infection by affecting the parasite, as in onion smut, where temperature affects spore germination. In other cases, infection is determined by the environment modifying the host, creating lowered resistance of the plant at either high or low temperatures moisture contents, or reactions. The resistance may be related to thickness and composition of cell walls or other effects. It has been shown by Thornton (71) that even the symbiotic development of legume bacteria may change to parasitism when conditions lead to decreased formation of carbohydrates within the plant. Rayner and Smith (57) refer to an observation that young roots of Calluna were found surrounded by an extensive development of bacteria in soils somewhat unfavorable to development of the plant. As a normal sequence of events, the attack of plant pathogens makes conditions favorable for the attack of certain remaining portions of roots by the saprophytic soil organisms.

It thus seems apparent that there is justification for the conclusion that sufficient organic substances come from roots to favor greatly the development of microörganisms about roots during plant development.

Organisms possessing great differences in capacity to attack plant tissues inhabit soils, most of the organisms being strictly saprophytic, however. There are several well established cases of root penetration by microörganisms, as with the legume bacteria and mycorrhiza fungi. Accompanying the specific legume bacteria, there is very frequently an organism called B. radiobacter (39, 34). Evidence also points to the fact that certain non-legumes may be quite generally penetrated by specific bacteria (79, chap. 4 and 62). A host of plant pathogens gain entrance to tissues. All of these cases are related to the characteristics of specific microörganisms, and such associations of microorganisms and plants are not common to the great majority of the soil population.

Root tissues which appear to be vigorous may not be entirely free from microorganisms. Jones (26) found fungi quite generally invading plant roots. Bacteria other than plant pathogens have also been detected frequently in roots. Perotti and his associates have found bacteria in the roots of a great variety of plants (47, 48, 49, 52, 53, 50, 51, 77). They were found diffused in the cortex and in the intracellular and intercellular spaces but not within the vascular system. In about 70 per cent of the cases studied, bacteria were found. It has not been assumed that the organisms are of pathogenic significance. It was assumed by Lominsky (40) that animal pathogens could gain entrance through wounds in plants and live within the tissues and also that all organisms penetrate root tissues. These results could not be duplicated by others (60, 8, 29), but Russell (60) observed that, subsequent to inoculation of plants with bacteria, many of the saprophytic forms persisted for a considerable period of time and even scattered some distances from the points of inoculation. Apparently some non-pathogenic bacteria can gain entrance into root tissues and persist in this environment, although most indications point to the fact that

only small numbers of bacteria occur in root tissues which have not degenerated.

A survey of these various observations leads to what seems to be a natural conclusion concerning some of the relationships between plant roots and soil organisms. Knowing that microörganisms develop where the supply of organic materials and environmental conditions are favorable, the development of organisms about roots seems to be controlled principally by the supply of organic matter. The sloughed off root caps and root hairs supply considerable food as the roots grow into the soil. Some amounts of exudations may appear at various regions of the root systems. Some cortical cells on growing roots may be sloughed off and superficial cells may become invaded by soil microorganisms. During a great portion of the period of plant development, most soil organisms are undoubtedly limited to regions outside of the roots. In response to organic materials coming from plant roots the microörganisms reach their maximum development in periods of advanced plant growth preceding extensive degeneration. Certain organisms may penetrate beyond the external cells, the depth of penetration being determined by the vigor of the root cells and the nature of the organisms in the rhizosphere. Some organisms may enter through openings in the superficial tissues.

The soil organisms should not be considered as passive individuals waiting for the death of roots before attack. They consume whatever materials are available at any time, the availability of root substances being affected by the resistance of cell membranes to microbial penetration and the occurrence of materials inhibitive to their development.

At the time of maturity and degeneration of the vegetative structures, many changes appear in the roots. As assimilation within the plant decreases, the root tissues become weakened through reduced food supply and they become the prey of soil organisms. The root hairs and fine roots disappear and the tissues of the larger roots become more deeply penetrated by microorganisms. The root system degenerates progressively until, at the time when the superficial portion of the plant is dead, there is little of the original root system which is in a vigorous condition free from microorganisms. All of this sequence of events may be greatly modified by particular effects such as extremely unfavorable environmental conditions or invasion of pathogens which may involve the entire root system.

Complete knowledge concerning the physiology and cytology of root systems during the course of growth, maturity, and degeneration will aid materially in explaining the changes which take place in microbial development. It is unlikely that observations at a single period would indicate the nature of the effects exerted by a plant, since the reactions of the microbial population undoubtedly closely follow the physiological changes in the root system.

SUMMARY

Experiments were performed to determine what differences exist between the abundance and activity of microörganisms on the immediate root surfaces of

plant roots, in soil close to the roots, and in soil farther removed from the zone of root development.   The results may be summarized as follows:

Microörganisms were much more abundant about roots.   The most pronounced increases were noted in the region of the root surfaces where the organisms were many times as numerous as in the soil close to the roots.

The general bacterial population and the bacteria of the Radiobacter group were affected by root growth to a much greater extent than the actinomyces and filamentous fungi.

Greater numbers of bacteria were found on roots of legumes than on those of non-legumes but the non-legumes exerted marked effects on the organisms.   No characteristic effect of legumes was observed except in the region of the root surfaces.   As many organisms were found in soils about roots of non-legumes as in soils about roots of legumes.

In general, the greater the distance from the region of extensive root development the smaller the number of bacterial inhabitants found in the soil.   Effects of roots on fungi and actinomyces were noted only in materials obtained from the root surfaces

Greater amounts of carbon dioxide were produced from soils obtained from regions of extensive root development than from soils obtained at any distance farther removed from the plants.   A close correlation appeared between the amounts of carbon dioxide produced by the soils and the abundance of bacteria detected in the samples.

Nitrification was most active in soils obtained from regions of maximum root development.

An explanation of the relationships between soil microörganisms and plant roots is advanced, which is based upon these observations, those presented in previous reports of this series of papers, and numerous reports of other related material.

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SOME INFLUENCES OF THE DEVELOPMENT OF HIGHER PLANTS UPON THE MICROÖRGANISMS IN THE SOIL: V. EFFECTS OF PLANTS UPON DISTRIBUTION OF NITRATES

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The nitrate ion in the soil solution commands particular attention because it is of primary importance in the development of most cultivated plants. It frequently occurs in soil in amounts insufficient for maximum plant growth and is supplied in the form of fertilizers only at considerable expense and labor. The fact that nitrate may be the end product of certain bacterial reactions, and is further susceptible to other modifications by soil organisms complicates the study of the availability of the nitrogenous resources of the soil for plant development.

Since nitrates generally originate through biological action, the amounts of nitrates in soils are frequently considered to reflect the degrees of microbial activity in these soils. There is also little question that in humid regions the soils that are rich in nitrates are among the most fertile. Burd (3) considered that the nitrate content of uncropped soils was the most valuable single criterion for appraising their crop-producing power.

There are, however, so many factors which affect the nitrate content of soils that isolated determinations in uncropped soils and in soils planted to different crops at various stages of development give no true representation of either the biological activity, the rate of nitrate formation, or the capacity of the soils to support plant growth. Stewart (24) found no nutrient ion in the soil solution affected more by cropping than was nitrate. In the soil solution of unplanted soil, nitrate was more abundant than any other nutrient; other plant nutrients as well as nitrate were lowered by cropping but nitrate more than any other.

Influence of plants upon soil nitrates.—The depression in nitrate content due to plant growth has been repeatedly observed and needs little comment other than mention of the fact that differences have been observed under different plants and the same plant at different stages of development (3, 4, 6, 7, 8, 9, 13, 14, 17, 24). Most of the removal of the nutrient ions occurs during the stages of extensive vegetative development when the plants make their greatest absorption of nutrients (10). During the late stages of development, many plants decrease in mineral nutrients and apparently a portion of the previously absorbed substances returns to the soil (4). Subsequent to death of the plants the nitrate content of soils tends to rise again (4, 24).

There are differences in the effects of specific plants upon soil nitrates. King and Whitson (12) considered that plant growth favored nitrification since the sum of the nitrogen in the crop and the nitrate of the soil supporting the crop was greater than the nitrogen in the nitrate of fallow soil. Russell (17) found that during late summer and early autumn the fallow land was richer in nitrate even after allowing for the nitrate taken up by the crop. Certain plant characteristics observed by Lyon, Bizzell, and Wilson (13, 14, 28) could be interpreted to explain these differences. It was observed that grass and wheat tended to depress nitrate accumulation whereas corn favored nitrate formation during the early stages of development. The nitrate content of soils previously grown to legumes maintained a higher level than the nitrate content of soils supporting certain non-legumes. The composition of the organic residues of these plants varied greatly and it seems likely that the specific differences exerted by the plants were due to a large extent to the differences in the plant residues. These residues were decomposed by microörganisms and if they contained appreciable amounts of nitrogen the result was favorable to the processes leading to nitrate accumulation. If the residues were low in nitrogen the assimilation of nitrogen by the microörganisms was sufficiently great to cause a depression in the rate of nitrate formation.

It seems likely, therefore, that at least two factors may be active in lowering nitrate concentration about roots: the absorption by plants and the absorption by microörganisms, the extent of the second being determined by the amount and nature of the organic materials put at the disposal of the microörganisms by the plant.

Nitrates in fallow soils.—Where plants are not developing, there are numerous factors exerting marked effects upon the distribution of nitrates. Temperature, rainfall, soil texture, amount and composition of the soil organic matter, soil reaction, and abundance of inorganic soil constituents are determining factors (1, 17). Almost invariably nitrates accumulate in fallow soils, and part of the increased fertility resulting from fallowing is to be ascribed to this change. The course of change in amount of nitrates in a fallow soil is not entirely in one direction, however. The fact that nitrates enter solution readily and are not appreciably retained by the soil colloids explains why nitrates migrate extensively in soils. Under the influence of heavy rainfall, nitrates pass into the deep regions of the soil and upon such occasions the quantity of nitrates may be greater in the subsoil than in the surface soil. Under conditions of rapid evaporation at the surface the nitrates may again rise with the capillary water (7, 8, 18, 19). One of the important rôles played by cover crops is the absorption of nitrates from the soil solution during periods when they might otherwise be lost from the soil in leaching waters, if no plant cover existed.

Variability in distribution of nitrates in soils.—These various factors and probably others create a lack of uniform distribution of nitrates in soils. Waynick (26) observed a pronounced variation in nitrate content in soils even

lacking vegetation.   In 81 samples of surface soil taken from an area 50 feet in radius he observed variations from 1.0 to 4.5 mgm. of nitrate nitrogen per 100 gm. of soil.   The 81 samples of subsoil varied from 0.3 to 2.2 mgm. Prince (16) observed similar degrees of variation.   The variation was greater in soils which had been treated with animal manure than in untreated soils. More recently Thornton and Gray (25) noted that the nitrate content of un-planted soil not only is different from place to place but changes within very short intervals of time.   Within 2 hour intervals such extreme changes were observed as from 35 to 14 p.p.m. in one case and from 26 to 50 p.p.m. in another. There was even some suggestion of diurnal fluctuations.

The degree of variability created by plant development seems to have com-manded little attention although it is acknowledged that considerable modifi-cation may be created by plants.   Hoagland (11) states the following:

It is, perhaps, not entirely accurate to picture the soil solution of an entire mass of soil gradually becoming reduced in concentration   Considering the plant as a whole, however, the supply of mineral elements would decrease as growth proceeds, if the mass of available soil were limited in amount, or if root growth ceased.

In unlimed soil, Blair and Prince (2) found more nitrate nitrogen at a dis-tance from the corn plant than near the base of the stalk.   In limed soils little difference was noted.   It was concluded that the difference between the limed and unlimed soils was due to the more limited root systems of the plants in the unlimed soils.

EXPERIMENTAL PROCEDURE

The results reported herein are concerned with the degree to which plant development affects the distribution of nitrate nitrogen in soils.   Soils which were used for studies of the influences of plant development upon the soil micro-organisms (20, 21, 22, 23) were observed for nitrate content.   The determina-tions were made over a period of 2 years.   During the first year comparisons were made between the contents of nitrate nitrogen in soil about plant roots and in soil supporting no plant development.   Certain biennials of this planting were continued for study during the second year and a newly planted crop was also used.   Determinations were carried out on soils about these plants at different distances from the region of massive root development.   Nitrates were determined by the phenoldisulfonic acid method, and the results are reported on the basis of the dry soil.

EXPERIMENTAL RESULTS

The results shown in tables 1, 2, and 3 show quite definitely that the nitrate nitrogen was much less abundant in soil taken from the vicinity of root de-velopment than in soil devoid of roots.   These experiments were conducted in the field and consequently the numerous climatic factors as rainfall, evapo-ration, and fluctuations in temperature exerted their uncontrolled influences on

the soils.　There appears to have been no regular course of change in the nitrate content of the unplanted soil during the first year but in the second year (see table 3, periods of 375, 399, and 456 days) there was a progressive increase in nitrate content.　The number of observations is, however, too limited to be of appreciable importance regarding the seasonal change in nitrates in unplanted soil.

In tables 1 and 2 lines are drawn between the columns of data to indicate the division between living and dead plants.　The potato plants died between the sixty-third and eighty-sixth day of sampling, the oats between the eighty-

TABLE 1

Influence of root development of potatoes, oats, and beans upon nitrate content of soil

PERIOD FROM TIME OF PLANTING	NITRATE NITROGEN IN SOILS—MGM PER 100 GM OF SOIL						
	Unplanted	Under potatoes	Loss due to growth of potatoes	Under oats	Loss due to growth of oats	Under beans	Loss due to growth of beans
days							
44	0 76	0 53	0 23	0 41	0 35	0 41	0 35
63	1 74	0 46	1 28	0 55	1 19	0 40	1 34
86	1 14	1 64	−0 50	0 83	0 31	0 69	0 45
138	0 91	1 86	−0 95	1 13	−0 22	0 62	0 29
173	1 00	1 23	−0 23	0 62	0 38	1 03	−0 03

TABLE 2

Influence of root development of corn and rape upon nitrate content of soil

PERIOD FROM TIME OF PLANTING	NITRATE NITROGEN IN SOILS —MGM PER 100 GM OF SOIL				
	Unplanted	Under corn	Loss due to growth of corn	Under rape	Loss due to growth of rape
days					
44	0 76	0 64	0 12	0 44	0 32
63	1 74	0 37	1 37	0 53	1 21
86	1 14	0 41	0 73	0 41	0 73
138	0 91	0 55	0 36	0 66	0 25
173	1 00	0 52	0 48	2 13	−1 13

sixth and the one-hundred and thirty-eighth day.　The beans and corn were killed by frost just previous to sampling on the one-hundred and seventy-third day.　It is quite clear that the roots of the potato plant exerted no depressing effect after death; a pronounced increase in nitrate is observed however at these last periods.　The effects of the other plants are not consistent. In the 42 periods of study of soils during which plants were living there are only two periods where there was less nitrate in the unplanted soil.

It is not possible to draw definite conclusions regarding the periods of greatest removal of nitrate.　Since the soils were not in closed containers the amounts

of nitrate in the unplanted soil do not indicate the amounts of nitrate that were formed; appreciable quantities were undoubtedly moved about outside of the region of sampling.   It is interesting, however, that most of the plants were in the stage of extensive vegetative development at the sixty-third day period, the time when the maximum difference between unplanted and planted soils appeared during the first year.   The sweet clover plants were comparatively small at this stage.   The lowest nitrate content under this plant occurred at the 173-day period, which was the period of greatest vegetative growth.   Much more regular changes in response to plant development would undoubtedly be noted if soils were confined in closed containers.

Table 4 records observations made in soils about three biennials during their second year of development.   It is apparent that the nitrates in soils supporting no plants are consistently more abundant than in soils either close

TABLE 3

Influence of root development of table beets, mangel beets, and sweet clover upon nitrate content of soil

PERIOD FROM TIME OF PLANTING	NITRATE NITROGEN IN SOILS—MGM. PER 100 GM OF SOIL						
	Unplanted	Under table beets	Loss due to growth of table beets	Under mangel beets	Loss due to growth of mangel beets	Under sweet clover*	Loss due to growth of sweet clover
days							
44	0 76	0 41	0 35	0 47	0 29	0 40	0 36
63	1 74	0 38	1 36	0 32	1 42	0 70	1 04
86	1 14	0 58	0 56	0 51	0 63	0 59	0 55
138	0 91	0 39	0 52	0 46	0 45	0 64	0 27
173	1 00	0 34	0 66	0 17	0 83	0 08	0 92
375	0 69	0 53	0 16	0 83	−0 14	0 28	0 41
399	1 88	0 54	1 34	0 57	1 31	0 57	1 31
456	3 11	0 93	2 18	1 09	2 02	1 20	1 91

* For sweet clover the sampling periods are 25, 44, 67, 119, 154, 356, 380, and 437 days

to the roots or a short distance from the main root systems.   The differences between the soils 15 cm. distant and those close to roots are not consistent, suggesting that the absorption systems of the plants kept the nitrate content to a fairly low level in these zones even during the second year of development. In all three cases, at the time of the final sampling, nitrates were more abundant adjacent to the roots than a short distance away, possibly a significant change coinciding with degeneration and decomposition of root materials near the end of their extended development over 2 years.

The results in table 5 are more definite and informative, since more regions of sampling are involved.   In the cases of mangel beets, differences are apparent at the three regions of sampling at the 66-day period.   At the 113-day period the root system created similar degrees of removal to the distance of 8

TABLE 4

Distance through which root development exerts its effects upon the nitrate content of soils—second year growth of biennials

PERIOD FROM TIME OF PLANTING	NITRATE NITROGEN IN SOILS—MGM PER 100 GM. SOIL		
days	Unplanted	15 cm. from main root*	Close to main root
Table beets			
375	0 69	0 59	0 53
399	1 88	0 57	0 54
456	3 11	0 52	0 93
Mangel beets			
375	0 69	0 57	0 83
399	1 88	1.11	0 57
456	3 11	0.79	1 09
Sweet clover			
356	0 69	0 20	0 28
380	1 88	0 50	0 57
437	3 11	0 96	1 20

* For sweet clover the soil was sampled at 31 cm distant from the main root system.

TABLE 5

Distance through which root development exerts its effects upon the nitrate content of soils—first year of a planting

PERIOD FROM TIME OF PLANTING	NITRATE-NITROGEN IN SOILS—MGM PER 100 GM SOIL			
days	40 cm from main root	23 cm. from main root	8 cm from main root	Close to main root
Mangel beets				
66		4 36	3 60	3 20
113	1 20	0 44	0 35	0 38
Beans				
66		2 59	1 55	0.89
113	1 80	1.09	0 94	0 63
Corn				
66		1 37	0 50	0 67
113	1.37	0 91	0 36	0 56

cm. from the roots´and greatly lowered the nitrates at a distance of 23 cm., but much higher quantities were found at 40 cm. distant.

The more limited root system of the bean plants brought about less disappearance of nitrates at a distance from the region of the origin of the roots. In both periods there is a progressive decrease in nitrates the nearer the soil is to the central root system. However, the plants appear to exert an influence to the distance of 23 cm.

The effects of corn are quite pronounced at a distance of 8 cm. at the first period and definitely extend to the distance of 23 cm. at the second period. The great variation in root systems of different plants undoubtedly modifies the distribution of nitrates in different ways. The extent of the root systems varies, the amounts of absorption at different periods are unlike, and the quantity and quality of the root residues create different responses in microbial activity.

DISCUSSION

It seems evident that plant development may greatly modify the distribution of nitrate nitrogen in soil, at least under certain conditions of plant growth. Where plants are quite completely isolated from one another the root systems fail to bridge the gap between and there develop zones from which extensive removal takes place through absorption by roots and other agencies. At a distance from these regions there is less removal by plants. A more dense stand of plants would tend to eliminate this variation in the lateral distribution, since the network of roots would permeate the soil quite completely at the advanced stages of development. The density of the stand that would bring nitrates to a somewhat uniformly low level would depend upon the extent of the absorptive system, which in turn would differ with the stages of development of any one plant, would vary with different kinds of plants under any one set of conditions, and would further be altered by climatic and soil conditions.

Accompanying the influence of root absorption there is the influence of microbial absorption of nitrates about roots during the decomposition of root residues and exudation products (14). These effects are presumably not the same under all plants or at different periods of development of a single plant. At certain periods at least, root absorption is much the more important factor. The root residues of sweet clover should presumably be somewhat richer in nitrogen than the residues of certain of the non-legumes which were used. However, the lowest content of nitrates in soils was observed under the sweet clover at the stage of its maximum vegetative development (173 days).

The vertical distribution of nitrates is undoubtedly also affected by plant development. In humid regions the effects at appreciable depths might not be as pronounced as the effects near the surface, since nitrates are concentrated in the superficial regions. In semi-arid regions the changes at lower levels might be more striking.

The observations of Blair and Prince (2) indicate that treatment of soil with lime so modified root development that more complete nitrate absorption took

place.   From the results of Weaver, Jean, and Crist (27) it is very apparent that root systems are susceptible to great modifications by fertilizer treatments, differences in texture and structure of soils, as well as differences in amount and distribution of moisture.   These numerous factors affecting root development undoubtedly lead to effects upon the removal of nitrates from the soil.

The decrease in nitrate concentration about roots can be interpreted as indicating that certain other ions are likewise depleted (3, 4, 5, 6, 24).   Exception may appear with phosphate and bicarbonate, the phosphate occurring in low concentrations whether or not plants develop and the bicarbonate definitely increasing about roots (6, 15).   The final balance as a result of plant growth shows a pronounced decrease in electrolytes (10).   It seems likely that such disappearance of nutrient ions would depress microbial activity, since it is generally conceded that the addition of fertilizer salts to soils of humid regions favors microbial development.   However, one cannot conclude that, since nitrate concentration is lower about plant roots, microbial development is also decreased.   In fact, very pronounced favorable effects are exerted upon the growth and activity of the soil microbes (20, 21, 22, 23).   It seems justifiable to conclude, therefore, that some other effect than the change in nitrate concentration exerts the dominant influence upon the microörganisms to cause their increased development.   It has been previously concluded that organic exudations from roots and degenerating root parts are of major importance. Other changes such as modified circulation of air and moisture probably lead to some of the responses in the organisms.

Likewise, the proportional amounts of nitrates about roots of developing plants compared with the amounts in soils at a distance from roots give no suggestion of the rates of nitrification in these soils or the potential rates with which nitrates would accumulate if the plant factor was eliminated at any time. In fact, in many instances, nitrate formation progresses more rapidly in soils taken from the root zone than in soil devoid of roots even though the soil which supports the roots contains very small amounts of nitrates at the time the soils are removed from the field (21, 22, 23).

It may be concluded that the amounts of nitrates in soils supporting plant development are of little use as indexes of either the activity of the soil organisms or the fertility of the soils.   Plant growth as well as other agencies may create great variations in the distribution of nitrates in soils, and consequently [in the words of Burd (5)]"   . . .   the large variation in nitrate content frequently observed in samples drawn within short distances of one another in the field may be without significance in terms of crop production."

SUMMARY

Determinations of the abundance of nitrates in soils about roots of developing plants and in soils free from roots indicate that, under field conditions, plants greatly lower the nitrate content of soils.

The degrees of removal of nitrates differ with the stages of growth of the plants.

The nitrate contents of soils at different distances from the center of root development are different.   Greater amounts of nitrates occur at a distance from the absorption system of the plant.

It seems unlikely that the modification of the nitrate content by plant development is a factor of importance in bringing about the acceleration in microbial activity which has been observed to accompany plant growth.

The results are discussed in regard to the influence of plant development upon variation in nitrate content of soils and the relationships between nitrate content, soil fertility, and microbial activity.

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BOOK REVIEWS

Handbuch der Pflanzenernährung und Düngerlehre, vol. I. Edited by F. HONCAMP. Julius Springer, Berlin, 1931. Pp. xv + 945, figs. 90.

This is the first volume of the Handbuch, devoted to plant nutrition. In preparing this volume, the editor had the assistance of noted authorities in their several fields. The major topics dealt with by the authors include a historical review of the evolution of our knowledge of plant nutrition; the proximate and ultimate composition of plant substances; the cycle of plant nutrients; the physiology of plant metabolism; the soil as a site and reservoir of plant nutrients; the so-called laws of crop yields; water culture and pot experiments; field experiments; the interpretation of fertilizer experiments; and the determination of the plant food requirements of soils. As indicated by this outline, the authors have made a very ambitious effort to review rather exhaustively the field of plant nutrition. The plant physiologist, the student of soil science, the agronomist, and the agricultural chemist will find in this work a wealth of information systematically arranged and discussed. The very extensive bibliography covers most thoroughly the writings of German investigators in the field of plant nutrition. At the same time, the references to the corresponding scientific literature of other countries are almost as complete.

Chemistry for Students of Agriculture and Home Economics, First Edition. By ROBIN CHARLES BURRELL. McGraw-Hill Book Company, Inc., New York and London, 1931. Pp. xviii + 459, figs. 77.

Chemistry is playing an increasingly important part in the training of students in agriculture and allied subjects. Soils and their management, farm manures and amendments, and particularly commercial fertilizers, represent materials whose chemistry is now better understood. Insecticides and fungicides, preservatives and antiseptics, plants and their derivatives, and animals and their derivatives are economically significant largely because of their chemical make-up. It is obvious, for this reason, that the author was justified in stating in the preface that "training in chemistry for students who do not intend to become specialists in this field of knowledge, must be designed simply to lay a broad general foundation. Many of the individual specific facts of the chemistry of tomorrow will be quite different from those of the chemistry of today. Yet these students as intelligent, wide-awake citizens, taking an active part in the world's work, will want to be able to understand the most recent discoveries about plant growth, the chemical control of insect pests and plant diseases, and the scientific feeding of animals and human beings. They

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will want to be able to appreciate the importance of chemistry in industry and the world problems that chemical discoveries often create."

The principal topics dealt with in the book include: fundamental principles of general chemistry; analytical and synthetic chemistry; organic chemistry; biological chemistry; and chemistry and the world's work. Under the topic "Biological Chemistry" the author deals with the fats and related substances, the carbohydrates, the proteins, the chemical and physical nature of living matter, the chemistry of plants, and the chemistry of animals. Under the topic "Chemistry and the World's Work" there is a discussion of chemistry in the home, chemistry in agriculture, chemistry in industry, and chemistry in problems of social welfare. There are five appendices, a glossary, and an adequate index.

The Soil and the Microbe. By SELMAN A. WAKSMAN and ROBERT L. STARKEY. John Wiley & Sons, Inc., New York, 1931. Pp. xi + 260, figs. 85.

The authors have appropriately dedicated their book to Sir John Russell, Director of the Rothamsted Experimental Station in England and widely known authority on plant nutrition, soil chemistry, and soil microbiology.

A conception as to the contents of the book may be had from the authors' statement in the preface. They say: "Our knowledge of the soil microbes and their rôle in soil processes and plant growth has developed in the last fifty years. However, a large body of information has since accumulated which enables us to construct a clear picture not only of the microscopic population of the soil, of its numerous physiological reactions, but also of the relation of these processes to the origin and formation of soil, to the cycle of elements in nature and to plant nutrition." The major topics dealt with by the authors consist of the soil and the plant, the microbe and its activities; the soil population and its distribution; the rôle of microbes in the distribution of organic substances in the soil; the transformation of nitrogen by soil microbes; the transformation of mineral substances in the soil through direct or indirect action of microorganisms; the interrelation between higher plants and soil microorganisms; modification of the soil population; and importance of microbes in soil fertility.

The undergraduate as well as the advanced student will find in this book a summary of pertinent facts on the subject of soil microbiology. The authors' intimate knowledge of the field assures us of the accuracy of the statements made by them and of the reasonable completeness of the information given by them. The book should stimulate an interest in an important part of the broad field of soil science and should offer to the instructor a textbook of the type not hitherto available.

Soil Management, Second Edition. By FIRMAN E. BEAR. John Wiley & Sons, Inc., New York, 1931. Pp. v + 412, figs. 58.

The purpose of the author in writing, and later revising, his book is well

indicated in the preface to the second edition.   He says: "The purpose of this book is primarily that of acquainting the student with the applications of those scientific facts and principles that are of use in planning constructive systems of soil management and increasing the productive capacity of soils."   The 26 chapters of the book are grouped under five larger divisions, designated, respectively, as Requirements of Crops; Characteristics of Soils; Utilizing Soil Resources; Conserving Soil Resources; and Supplementing Soil Resources.

While it is obvious that it is not possible to offer an exhaustive treatment of the subject within the scope of 400 pages, the author has been successful, nevertheless, in effectively organizing a large mass of facts and deductions and of presenting it in very readable form.   The revised edition should prove as widely useful as is the first edition to beginners as well as to fairly advanced students of soils and soil fertility.   The book will also be found to have a distinct value for reference purposes.

J. G. LIPMAN.

RELATION OF pH DRIFT TO MOISTURE CONTENT AND BASE HELD IN SOILS

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This paper deals with a study of the pH values and their drift, in soils at various soil-water ratios as affected by exchangeable bases and water content of soils, when measured by the quinhydrone electrode.

There has been much discussion concerning the determination of hydrogen-ion concentration of soils by the quinhydrone electrode. In certain soils a decided drift of pH with time is found to occur when determinations are made by this method. The International Society of Soil Science (4) has studied the problem, the studies being made in part on account of the report by Kühn (9), who suggested that soil investigators give up the quinhydrone electrode and use the indicator method. Kühn finds many soils whose pH value as measured by the quinhydrone electrode will vary as much as 0.5 units in one and a quarter minutes. Biilmann and Tovborg-Jensen (1) found no appreciable drift of potential between the readings of one-half minute, fifteen minutes, and four hours after the addition of quinhydrone. Heintze and Crowther (7) suggest that an active form of manganese dioxide in intimate association with soil colloids might cause an error in the determination of soil reaction by the quinhydrone electrode, due to the reduction of manganese dioxide to manganous hydroxide. McGeorge (10) concludes that the accuracy of the quinhydrone electrode for the determination of soil reactions is greatly affected by the presence of small amounts of manganese dioxide; but does not condemn the method, as the drift shown by the potentiometer is sufficiently rapid to warn the analyst. Brioux and Pien (2) found abnormal values by the quinhydrone electrode for soils overlying chalk. Among others Heintze and Crowther (7) report that Christensen and Tovborg-Jensen (3) find that laterite soils give high pH values by the quinhydrone electrode. It is well known that the quinhydrone electrode is not accurate for solutions more alkaline than about pH 8.5.

That soils possess the capacity to hold and exchange bases has been well established by the work of Gedroiz (6). He also shows that soils holding a particular base exhibit notably individual physical characteristics, giving tables which show that air-dry soils saturated with various bases will take up water

[1] Contribution no. 158, department of chemistry.

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and increase their volume by various amounts. Thus soils saturated with sodium will swell 47 per cent while soils holding ammonium, potassium, calcium, aluminum, and ferric iron will swell 24, 19, 17, 6, and 4 per cent, respectively. The amount of water held by the various soils at complete saturation with water in grams per 100 gms. soil in sodium 106, ammonium 78, potassium 73, calcium 71, aluminum 63, and iron 51. Smolik (13) found that soil samples saturated individually with sodium, calcium, magnesium, potassium, and ammonium held water in decreasing order of magnitude. The authors also found that the base held by a soil had considerable effect on its capacity to hold water. These variations are taken up under the heading of "The Soils Used."

In studying the problem of potential drift the International Society of Soil Science (5) reports that of 28 soils studied 20 gave pH values obtained by the quinhydrone electrode comparable to those obtained by the hydrogen electrode. The other eight soils showed a decided drift of potential when the quinhydrone electrode was used. A study of the report shows that the drift probably consists of two parts: first, a rapid drift for about 60 seconds after the addition of the quinhydrone and, second, a slower drift over a longer period of time. Fifteen minutes was the maximum time for which the drift was studied.

THE PROBLEM

With the facts of base exchange, altered physical characteristics of the soil as governed by exchangeable base, and drift of pH of soils obtained under various conditions the authors decided to investigate the effect of exchangeable base on potential drift. The bases used in treating the soils were sodium, potassium, ammonium, hydrogen, calcium, magnesium, aluminum, and ferric iron. In conjunction with these studies it was decided to make the reaction determinations on soils dried over calcium chloride and on soils saturated with water by keeping them over water. The measurements were made at soil-water ratios of 1–1, 1–2.5, 1–10, and 1–100. The ratio of 1–2.5 is probably the one most commonly used in soil investigations.

APPARATUS USED AND TECHNIQUE

In general the apparatus and technique used is that given by the International Society of Soil Science (4). The platinum electrode used was heavy platinum wire of 1.5 mm. diameter, which was somewhat flattened out at the end or bent over. Thus a strong electrode is obtained of about 1 sq. cm. surface without mercury contact, and the frailty of a glass tube is avoided. Morgan, Lammert, and Campbell (11) recommend an electrode of at least 1 sq. cm. surface. The electrode vessels used were glass vials 1.5 cm. in diameter by 6 cm. in length. The potentiometric outfit used balances current resistance drop taken from an Edison cell against the E.M.F. of the electrode system, the E.M.F. being measured by a voltmeter readable to 0.001 volt. The galvanometer used as a null point indicator was sensitive to 0.025 of a micro ampere. The soil and water were mixed and then the quinhydrone was added. The

mixture was shaken vigorously for a few seconds before the electrode was inserted. The mixture was then stirred with the electrode for about 10 seconds and the first reading taken 30 seconds after the addition of the quinhydrone. To eliminate the effects of sedimentation the mixture was always restirred immediately before a reading was taken. Measurements were made in the presence of air. Numerous measurements were made under a stream of hydrogen, the hydrogen being led to the surface of the soil-water suspension and not into it. Thus contact with the air and adsorption of CO_2 were avoided but the suspension was not stirred by the hydrogen, as it is considered that a stream of hydrogen through the soil will drive out the carbon dioxide naturally occurring therein. Under these conditions the drift was approximately the same as in the presence of air. The water used in making the dilutions was CO_2 free, being distilled from a mixture of sodium hydroxide and potassium permanganate. The first portion of the condensate was discarded.

THE SOILS USED

Six surface soils were selected for this work. These soils, which have been described in detail by Perkins and King (12), are mineral soils obtained in the southeastern and central eastern portions of Kansas. The original soils are somewhat acid in nature. The finer portions only of the soils have been used, a rough separation having been made by sedimentation so that only the portions most active in base exchange were retained. The soils were treated with normal chloride solutions of sodium, potassium, ammonium, calcium, magnesium, ferric iron, and aluminum, and twentieth normal hydrogen chloride. The treatments were continued until no further base exchange phenomena were evident. The soils were then washed with water and alcohol until practically free from excess electrolyte. That the soils treated as aforementioned are saturated with the various bases to the same degree is not maintained, as the unneutralized chloride solutions were used in exchanging the bases and there was a chance for hydrogen ions to be present as a result of hydrolysis when washing. However, the soils were saturated with the particular base to a far greater degree than natural soils would be.

The soils thus treated were air-dried for a period of six months and then subdivided, portions being placed in desiccators over water (in the presence of air) and other portions over calcium chloride. The desiccators were then placed in a room fairly well insulated against temperature changes and kept there until the weight of the soil indicated that equilibrium in regard to absorption or loss of water had been reached. A period of six months was required. The water-holding capacity of the soils as determined by adding the water loss of the desiccated soils to the moisture gained by the soils held in water vapor varied. The average water-holding capacity in per cent of desiccated soil for the soils treated with various bases was: Fe 22, H 23, Mg 25, Ca 30, NH_4 33, Al 33, Na 39, and K 41.

pH DRIFT STUDIES

The 96 samples of soil as prepared were used to study the potential drift of the quinhydrone electrode over a period of 15 minutes. Each determination was made in triplicate, and the last two were averaged and reported. Determinations were made at four soil-water ratios 1–1, 1–2.5, 1–10, and 1–100. As

TABLE 1

pH Values of soils treated with various bases

At indicated time intervals after addition of quinhydrone, averages for six soils, soil-water ratio of 1–1

BASE SOIL WAS TREATED WITH	MOIST SOILS						DRY SOILS					
	Time intervals in minutes						Time intervals in minutes					
	½	1	2	5	10	15	½	1	2	5	10	15
Na .	6 87	6 88	6 89	6 88	6 88	6 89	6 50	6 48	6 45	6 42	6 40	6 41
K .	6 80	6 80	6 79	6 75	6 74	6 73	6 46	6 47	6 48	6 49	6 49	6 50
Ca	6 39	6 43	6 44	6 46	6 48	6 47	6 31	6 31	6 30	6 30	6 30	6 30
Mg	6 34	6 33	6 34	6 32	6 27	6 24	6 11	6 12	6 13	6 17	6 17	6 19
NH₄	5 72	5 75	5 75	5 75	5 75	5 75	5 44	5 42	5 41	5 38	5 37	5 36
Al	4 42	4 40	4 36	4 33	4 32	4 30	4 25	4 18	4 17	4 17	4 14	4 14
Fe	3 59	3 58	3 53	3 46	3 44	3 42	3 16	3 14	3 13	3 12	3 10	3 08
H	3 71	3 70	3 69	3 67	3 66	3 65	3 25	3 24	3 23	3 21	3 17	3 17

TABLE 2

pH Values of soils treated with various bases

At indicated time intervals after addition of quinhydrone, averages for six soils, soil-water ratio of 1–2 5

BASE SOIL WAS TREATED WITH	MOIST SOILS						DRY SOILS					
	Time intervals in minutes						Time intervals in minutes					
	½	1	2	5	10	15	½	1	2	5	10	15
Na	7 11	7 13	7 14	7 15	7 15	7 15	6 89	6 86	6 86	6 89	6 87	6 87
K	6 92	6 91	6 90	6 86	6 82	6 78	6 67	6 67	6 67	6 67	6 67	6 66
Ca	6 64	6 63	6 61	6 57	6 61	6 57	6 39	6 39	6 39	6 38	6 37	6 36
Mg	6 20	6 19	6 19	6 17	6 11	6 08	6 37	6 39	6 39	6 39	6 36	6 35
NH₄	5 92	5 89	5 87	5 85	5 84	5 79	5 70	5 65	5 65	5 64	5 60	5 56
Al	4 30	4 27	4 22	4 19	4 14	4 10	4 16	4 12	4 12	4 12	4 11	4 08
Fe	3 93	3 92	3 76	3 68	3 63	3 57	3 39	3 37	3 37	3 32	3 27	3 24
H	3 76	3 74	3 71	3 66	3 62	3 59	3 36	3 36	3 34	3 30	3 25	3 20

all the six soils used gave similar curves the results have been averaged and reported in tables 1, 2, 3, and 4 for the various ratios. Thus each figure in these tables is an average of 12 determinations. In adding the water to the soil the weight of the air-dry soil was taken on the basis of the soil weight for the soil-water ratio.

From a study of tables 1 and 2 where the results of the studies of the soil-water ratios of 1–1 and 1–2 5 are reported, the ratios of lesser dilution and those most commonly used, it is not evident that pH drift is associated with any particular base at these dilutions, the greatest fluctuation between the $\frac{1}{2}$-minute and 1-minute pH readings is 0 07 units in the case of the dried aluminum soil

TABLE 3

pH Values of soils treated with various bases

At indicated time intervals after addition of quinhydrone, averages for six soils, soil-water ratio of 1–10

BASE SOIL WAS TREATED WITH	MOIST SOILS						DRY SOILS					
	Time intervals in minutes						Time intervals in minutes					
	½	1	2	5	10	15	½	1	2	5	10	15
Na	7 38	7 35	7 32	7 30	7 26	7 24	7 37	7 31	7 30	7 25	7 22	7 14
K	7 21	7 14	7 08	6 98	6 91	6 81	6 81	6 78	6 77	6 76	6 70	6 67
Ca	6 73	6 72	6 61	6 57	6 49	6 45	6 53	6 52	6 51	6 49	6 47	6 45
Mg	6 19	6 16	6 10	6 01	5 96	5 91	6 57	6 53	6 50	6 47	6 43	6 41
NH$_4$	5 93	5 87	5 79	5 76	5 70	5 64	6 10	6 08	6 03	5 96	5 86	5 81
Al	4 20	4 17	4 14	4 12	4 09	4 07	4 17	4 12	4 10	4 09	4 07	4 06
Fe	4 07	4 03	3 95	3 85	3 73	3 67	3 62	3 59	3 57	3 51	3 47	3 43
H	3 89	3 85	3 78	3 71	3 67	3 64	3 49	3 45	3 42	3 34	3 26	3 22

TABLE 4

pH Values of soils treated with various bases

At indicated time intervals after addition of quinhydrone, averages for six soils, soil-water ratio of 1–100

BASE SOIL WAS TREATED WITH	MOIST SOILS						DRY SOILS					
	Time intervals in minutes						Time intervals in minutes					
	½	1	2	5	10	15	½	1	2	5	10	15
Na	7 28	7 18	7 14	7 04	6 96	6 88	7 47	7 37	7 20	7 05	6 87	6 77
K	6 83	6 71	6 66	6 44	6 29	6 16	6 59	6 53	6 48	6 39	6 32	6 28
Ca	6 14	6 03	5 99	5 93	5 87	5 83	6 59	6 58	6 57	6 53	6 47	6 44
Mg	5 74	5 70	5 63	5 57	5 50	5 49	6 57	6 56	6 49	6 45	6 39	6 35
NH$_4$	6 02	5 87	5 76	5 65	5 56	5 52	6 43	6 38	6 31	6 14	5 96	5 87
Al	4 37	4 32	4 28	4 26	4 22	4 21	4 63	4 54	4 50	4 46	4 43	4 41
Fe	4 41	4 32	4 21	4 09	4 02	3 97	4 07	4 02	3 93	3 87	3 81	3 79
H	4 21	4 15	4 08	3 96	3 91	3 89	4 10	3 95	3 84	3 71	3 65	3 61

This is hardly a significant figure in soil work   The greatest drift for the 1–1, and 1–2 5 dilutions over the 15-minute period is 0 36 units in the case of iron. This would be considered significant if it were not for the fact that at the greater dilutions both sodium and potassium show greater fluctuations than does the iron.   The figures published by the International Society of Soil Science (4)

show that for several soils at the 1–2.5 dilution there is a pH drift of approximately 0.3 units from the $\frac{1}{2}$-minute reading to the 1-minute reading. No drift of potential on the soils that we have studied even approximates this value.
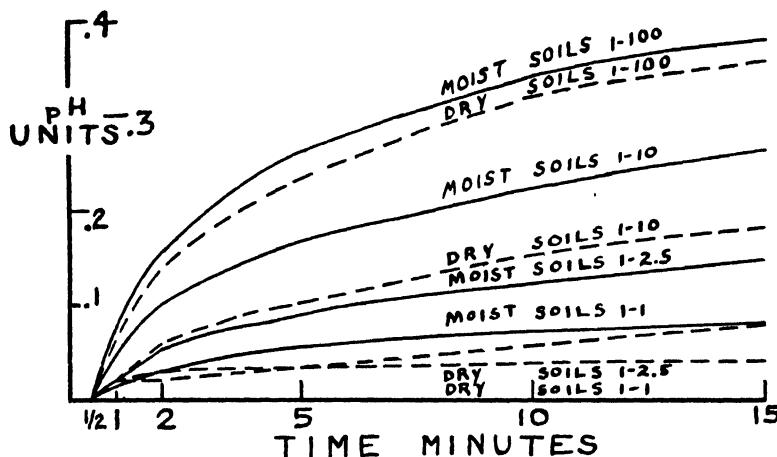
FIG. 1. DRIFT OF pH AT VARIOUS SOIL-WATER RATIOS

pH Units plotted against time
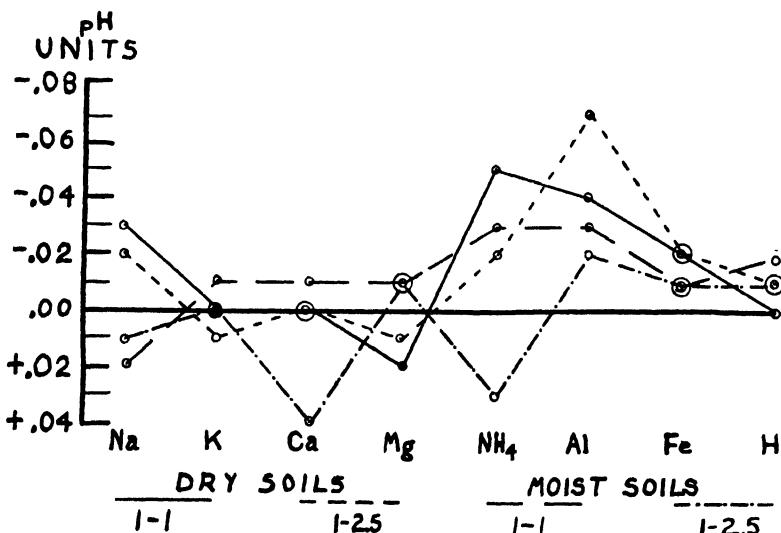
FIG. 2. DRIFT OF pH ONE-HALF MINUTE TO ONE MINUTE. SOILS TREATED WITH VARIOUS BASES. SOIL-WATER RATIO 1–2.5 AND 1–1

It is interesting to note that there is greater drift of pH in the case of soils that were saturated with water at the start than in the case of the dried soils. This is especially true for the drift at 2-minute and longer intervals. A study

of the tables will show the general trend of the drift for the dry soils as compared to the moist soils. The drift is emphasized by the curves in figure 1, which are drawn from the averages of each of the six soils treated with the eight bases. Thus each point in the curve shows the average drift of 48 samples. By averaging the results certain irregularities were smoothed over.

It should also be noted that the greater the degree of dilution the greater is the drift of pH value. This might be attributed to several things, among which is that in the more dilute soil suspensions there is less soil to act as a buffer against possible change in reaction.

It should also be noted that in some cases the drift of pH was observed over 18-hour periods. The figures obtained from these studies indicate that the curves are asymptotic and that the relative drifts shown at the 15-minute intervals are comparable to those obtained at the larger interval.

Curves drawn from the data herein presented showing the effect of dilution on pH value of the soils do not agree with the curves given by Perkins and King (12) for air-dry soils. However, it should be noted that there is good agreement between curves for the air-dried soils and the soils dried over calcium chloride. Discrepancies appear when one tries to match the curves for the moist soils with the curves for the dried soils.

CONCLUSIONS

At the commonly used soil-water ratios exchangeable base has no influence on potential drift (fig. 2).

The amount of water contained by the soil apparently influences the degree of pH drift, the moist soils having a larger drift than the dry soils (fig. 1). This is especially true for the 2-minute period and longer periods of time.

The drift of pH is variable with degree of dilution, the greater the dilution the greater the drift (fig. 1).

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FURTHER OBSERVATIONS UPON THE NATURE OF CAPILLARY RISE THROUGH SOILS[1]

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In a previous paper (10) the writer and Alfred Smith[3] reported that a greater and more rapid rise of water by capillary movement from a free water table might be expected when soil columns of a relatively large cross section were used as compared to columns of smaller size. No explanation of this observation was made at that time. Continued interest has resulted in further studies, undertaken partly in Stanford University and more lately under the authority of the Hawaii Agricultural Experiment Station. Although the reasons for the results reported in the previous paper are still subject to some speculation, the investigations have led to a better understanding of the nature of capillary rise through soil.

Except as noted, the observations reported deal entirely with the movement of liquids in soils or other materials when those materials are supported over a free surface of that liquid. The reported rise of water is always vertical.

EXPERIMENTAL

Observations with glass tubes

Although it is recognized that the minute, branching and circuitous capillary conduits in a soil mass differ widely from those in an idealized arrangement of spherical grains, a study of such material is often illuminating. Meinzer (8) discusses the nature of the pore space in such idealized packings and points out that the pore spaces through such a system are capillary tubes of essentially triangular cross section. As the pore space follows the surface of the grains it enlarges in area and then diminishes to its former value as critical elevations are reached. Since in any random packing of sand grains perfect arrangement cannot be obtained, it may be assumed that the effective pore space, in a significantly large cross section, is essentially continuous and of practically uniform dimensions.

Consequently, studies were made of the rate of rise of water in minute glass tubes when erected over water. These capillary tubes were drawn from care-

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fully cleaned glass tubes after heating in an oxygen flame. A heated section of less than 2 inches was quickly drawn to more than 4 feet. Short sections from the middle of this extended section were preserved for observation. From the method involved, it is evident that the minute tubes could not be perfect cylinders but necessarily are of varying radii. This effect, however, was not apparent in the observations. After each run the tube was reversed and a rise noted in the opposite direction. In no case did this reversal materially affect the characteristics of the rise. Precise observations of the rise of water through such systems are difficult. Although the probable error of a single observation of rise against time must be high, results for all tubes are consistent. Figure 1 illustrates the relation between observed capillary rise and elapsed time in a typical tube.
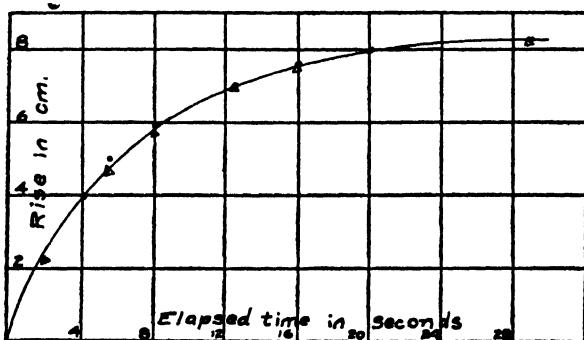
FIG. 1. RISE-TIME CURVE FOR GLASS TUBE OVER WATER

The shape of this curve (fig. 1), as well as the nature of the phenomena represented, indicates a curve of exponential form. Inspection and trial suggests the form:

$$R = K (1 - \epsilon^{-ct}) \qquad (A)$$

where R = rise in centimeters after t seconds, ϵ = the base of natural logarithms while K and c are constants, the first being the asymptotic value of R, while c is probably a function of the diameter of the tube as well as the characteristics of the liquid used.

For the tube used in figure 1, the constants, as a close approximation, are

$$R = 8.25 (1 - \epsilon^{-0.146t}) \qquad (B)$$

The closeness of the fit of equation (B) to the observed points is also indicated in figure 1, observed points being shown as solid dots, while computed points are enclosed in triangles.

The difficulty of obtaining an unquestioned evaluation of the friction loss due to turbulence during the early rise, prohibits the setting up of a differential

equation covering the relation between rise and elapsed time. Equation (*A*) can only be considered as an empirical approximation.

Observations with inert sands

Meinzer's (8) description of the nature of the capillary conduits in clean spherically grained material would indicate that the capillary rise of moisture through such material should follow some such law as that suggested in equation (*A*). Ottawa testing sand was used to test this assumption. This material is commercially available in only two classifications; one of these is the 20–30 grade, all the material passing a standard 20-mesh screen and being retained on the 30-mesh, and the other is unscreened. The grains are not perfectly spherical, as seen through the microscope, but nearly so. Although essentially clean, the material was washed in 10 changes of water with constant agitation. The last washing was with distilled water.
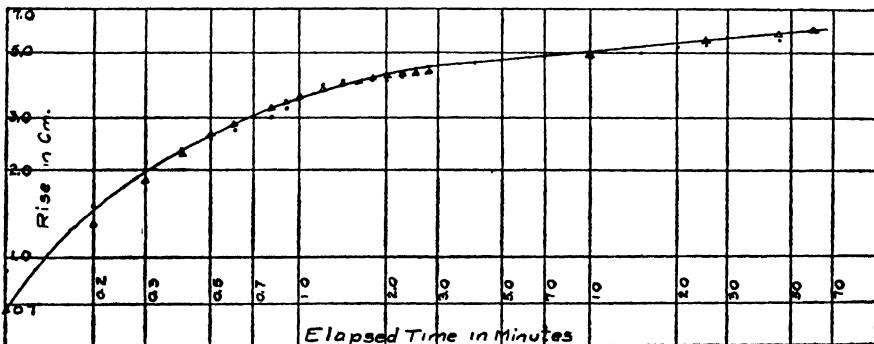
FIG. 2. LOG.-RISE LOG.-TIME FOR UNSCREENED TESTING SAND

Since a limited assortment of screens was available, only three separates from the unscreened material were possible. The screened separates were 20–35, 35–48, and 48–65. The mill screened, 20–30, separate added another lot. The washed, unscreened sand was also used.

These sands were carefully packed into 2-inch glass tubes fitted with brass screens at the lower end. Practically constant volume weight was obtained throughout each tube by the method already described (10). At least 10 columns of each material were used.

These columns were erected over distilled water, zero-hour being the time at which water in the sand mass had attained the elevation of the water in the reservoir. Elapsed time was taken with a stop watch. Frequent observa-,ions were made, especially at the beginning of each run, the time interval between observations during this period being one-tenth of a minute. This interval increased as the rate of rise was reduced. All sand columns were under observation for 60 minutes.

In general, the curves resulting from plotting log-rise against log-time showed the same general form. This form is illustrated in figure 2, which represents the observations taken on run 6 of the unscreened material plotted logarithmically. Here the initial arm of the curve is similar in form to the exponential curve noted for the glass tube. The curve does not reach an asymptotic value as might be expected, however, the second phase of the rise being parabolic for the period between 2.8 to 60 minutes. The parabolic nature of the upper part of the curve has already been noted, when soils are used, by McLaughlin (6) who derives empirical equations for rise against time in this phase of his observations. In the results reported in the previous paper (10) the parabolic nature of the curves continued for about 150 days. In general, the initial phase of the curve has not been studied.

Although there is no mathematical discontinuity in such curves as that illustrated in figure 2, there seems to be some evidence that two processes, acting simultaneously, are effective. One of these is of primary significance during the initial phase of the rise, whereas the other is evidenced by the long, slow rise in the second arm.

The equation

$$R = K (1 - \epsilon^{-ct})t^n \qquad (C)$$

where n = a constant and equals the slope of the straight arm, other symbols being as given, seems to fit these conditions. For small values of t the factor $(1-\epsilon^{-ct})$ dominates the rate; for large values of t the binomial becomes practically unity and the equation of the parabola remains.

When proper values are chosen for the constants, the computed rises for the given time periods fit the observed points with acceptable accuracy when clean sands are used, in view of the approximation mentioned for equation (A). Great precision of observation cannot be expected during the early readings when the rise is rapid and the interval short. Moreover, errors are seemingly exaggerated in this region because of the nature of the ruling used.

Suitable values of the constants for the curve in figure 2 give an equation:

$$R = 3.85 (1 - \epsilon^{-2.56t})t^{0.108} \qquad (D)$$

In this determination, and all that follow, n is the natural tangent of the slope of the second arm, K is the intercept of the second arm extrapolated to unit time, and c is the average value obtained by considering each of the observed points in turn, in view of conditions already established. Computed values for rise from equation (6) are shown in triangles in figure 2. Although the fit is far from perfect, it may be considered as a first approximation. It should be noted that the first observation, after 6 seconds of run, usually shows a greater rise than the computed value.

Equations for all other runs were determined in the same way. These equations may be summarized by listing the average value and probable error of each of the constants involved.

The results of these computations are given in the following:

Separate	Equation	
20–30	$R = 0.97 \pm 0.07 \, (1 - \epsilon^{-(6.18 \pm 0.34)t}) \, t^{0.113 \pm 0\,013}$	(E)
20–35	$R = 2.30 \pm 0.06 \, (1 - \epsilon^{-(3.54 \pm 0\,11)t}) \, t^{0.069 \pm 0\,005}$	(F)
35–48	$R = 2.40 \pm 0.05 \, (1 - \epsilon^{-(3.16 \pm 0.204)t}) \, t^{0\,061 \pm 0.005}$	(G)
48–65	$R = 4.41 \pm 0.09 \, (1 - \epsilon^{-(2\,07 \pm 0\,05)t}) \, t^{0\,129 \pm 0\,004}$	(H)
Unscreened	$R = 3.41 \pm 0.11 \, (1 - \epsilon^{-(2\,53 \pm 0\,17)t}) \, t^{0\,131 \pm 0.006}$	(I)

In general, the value of the coefficient increases with decreasing grain sizes, while the value of the constant factor in the exponent decreases in the same sense. Although the points as plotted in figure 3 show general trends as indicated in the foregoing, they depart widely from simple relationships. The
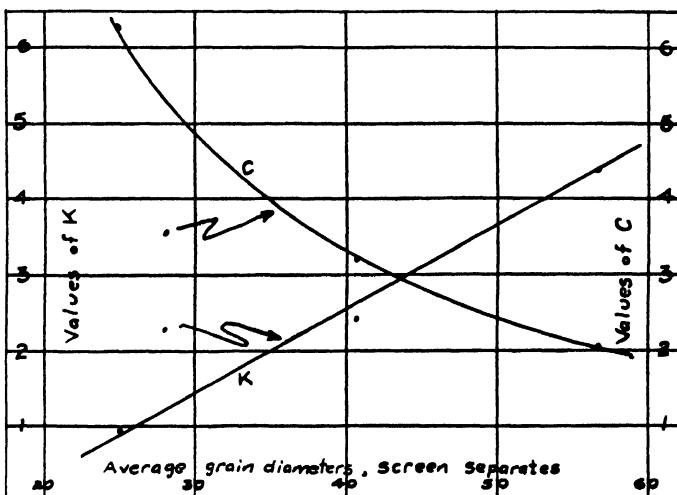
FIG. 3. RELATION BETWEEN CONSTANTS FOR TESTING SAND AND GRAIN-SIZES INVOLVED

lines drawn on figure 3 illustrate trends only and have little quantitative significance. Departures from simple trends are most pronounced in the largest hand-screened separates. Here the values for both constants indicate an average size much smaller than the average screen-size used in its separation. No information is available with respect to the distribution of grain sizes between the specified limits. If the normal unscreened material had been robbed of a large part of its 20–30 separate before shipment, the 20–35 separate as prepared in the laboratory would be rich in the finer sizes, and some such result as noted might be obtained.

Another significant conclusion from the equations for rise against time with inert materials is that the exponent of t is apparently independent of the grain size. Although these values range from 0.061 ± 0.005 to 0.131 ± 0.006, there is no consistent relation with grain size. Here the arithmetic

difference is about nine times its probable error. Although such a ratio is ordinarily enough to establish significance in a difference, the relatively large probable error attached to each term and the similarity of values at each end of the grain-size series seem to reduce the significance in this case.

However, this value seems somewhat dependent upon the physical conditions of the liquid, as may be noted when soils of such texture are used, that the second arm may extend for several days before ceasing to follow the parabolic law. When such a column is subjected to extreme diurnal changes of temperature, the first observation in the morning is consistently above its extrap-
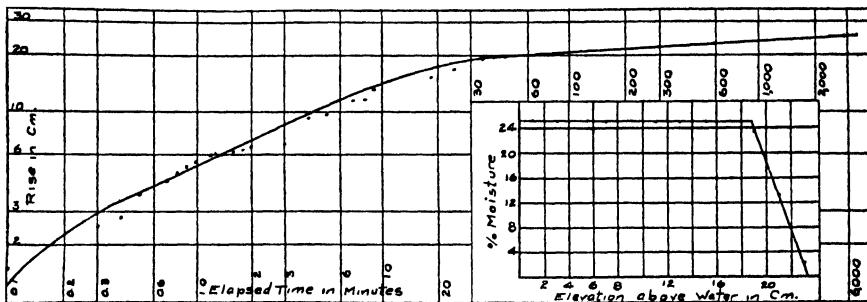
FIG. 4. LOG.-TIME LOG -RISE FOR FINE EMERY, TOGETHER WITH RESULTING MOISTURE DISTRIBUTION
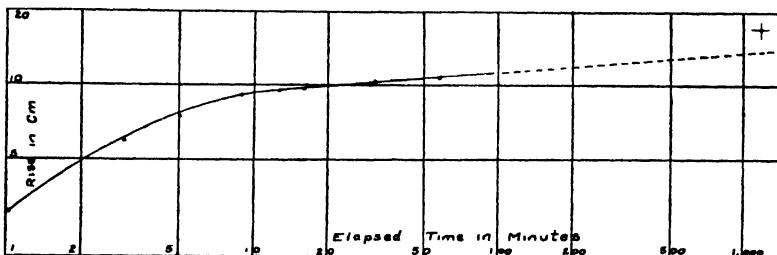
FIG. 5. LOG.-RISE LOG.-TIME CURVE FOR UNWASHED BUILDING SAND

olated position as determined by observations on the previous day. Although both surface tension and viscosity increase with lowered temperature, the effect of surface tension in this case seems more marked. This effect is illustrated by the last point in figure 5.

Observations with finely ground emery

The possibility of noting a change in the nature of moisture distribution at or near the point at which the rise began its distinctly parabolic aspect, led to the substitution of finely divided emery for sand. In commercial lots this material is described by the screen number of the smallest screen that will

allow all the grains to pass. No measure of the smallest grain is available, nor is the distribution of sizes known. Moreover, the grains are distinctly angular. Commercial emery is apparently coated with a thin film of oil. This material was removed by ether extraction by means of an Allihn condenser and a final flushing with clean ether.

This washed and dried material was packed into a 2-inch glass tube formed by cementing together the ground faces of glass sections, each 2 cm. high, with a thin layer of rubber cement. The assembled tube was reenforced by a wooden trough into which the tube was lashed. McLaughlin (7) reports a similar arrangement. With the brass sections used by McLaughlin, observations of rate of rise were impossible.

Observations of rise against time were made as usual, the results being presented in figure 4. Here the nature of the initial phase is distinctly different from the characteristic exponential form of the more uniform sand. In fact, a straight line might represent the observed points as well, or better, than the arbitrary curve in figure 4. Considerable difficulty was experienced with this material because little or no color change was apparent upon wetting. In fact, no rise above 20 cm. was evident through the glass, although upon disassembling, distinctly wet emery was encountered at 25 cm. In this case the straight arm is drawn by analogy. It is assumed that the rise was real, although not apparent through the glass, between 40 and 2,400 minutes, and that it followed the parabolic law during this period. The column was disassembled after 2,400 minutes. Each section was removed with a broad spatula and placed at once in a weighing bottle. Drying was accomplished as usual, at 110°C. The resulting moisture distribution is also shown in figure 4. Here all samples below 19 cm. showed essentially uniform moisture contents at the percentage subsequently identified as the saturation percentage. Greater elevations were characterized by continuously decreasing moisture contents.

Observations with soils

When soils are used the rather distinct and easily defined conditions described in the foregoing become much more complex. The size of grain involved and the distribution of grain sizes is no longer known with any exactness, whereas colloidal material may affect the results in many ways. Moreover, soluble material undoubtedly affects both surface tension and viscosity. Consequently, curves of rise against time are inconclusive and of difficult interpretation.

However, some of the characteristics of the curves from simple materials remain. They are all characterized by the parabolic arm in the second phase of the rise, and in general the slopes of these lines when plotted logarithmically are essentially the same. The time required for the inauguration of the parabolic arm is often very long, especially with fine-grained material. Moreover, all such parabolic curves eventually cease their simple relations and approach an asymptotic value. In general, the finer grained the material the more extended is the parabolic part of the curve.

When soils are used the initial curve described for sands as one of exponential character tends to approach the parabolic form suggested by the initial curve for emery.   The general form suggested in equation (C) no longer holds, since the coefficient of the exponent t becomes a variable.   In fact, with many soils the initial arm is practically straight for a considerable time, and then through a long curve assumes the characteristic parabola of the upper arm.
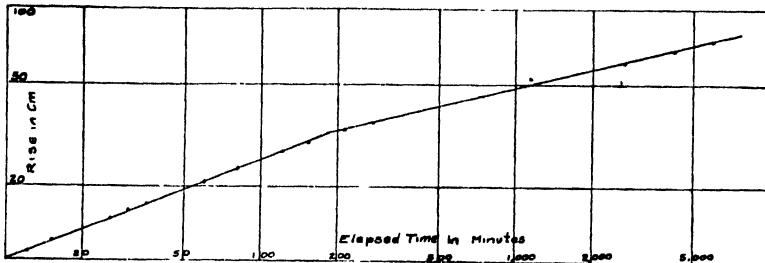
FIG. 6. LOG.-RISE LOG.-TIME FOR YOLO SANDY LOAM
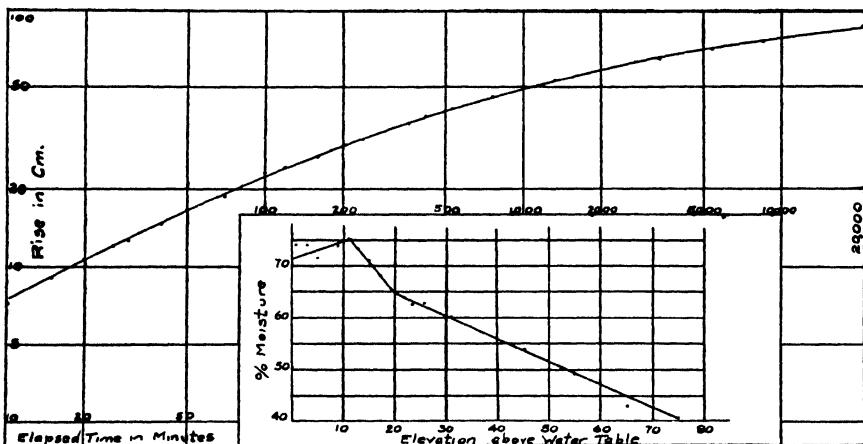
FIG. 7. LOG.-RISE LOG.-TIME CURVE FOR NATURAL KUNIA SOIL, TOGETHER WITH RESULTING MOISTURE DISTRIBUTION

The fineness of soil grain involved seems to be the dominating influence in determining the length of this initial arm of the curve.   When ordinary building sand was used, the parabolic section of the curve began at about 15 minutes. With Yolo fine sandy loam, less than one day was required, whereas with a fine-grained soil from the pineapple fields at Kunia (Oahu, Hawaii), no sign of parabolic rise was evidenced for four days.   Similar soil, exposed to a temperature of 1,100°C. for about five hours was so finely divided that the top of the 100 cm. tube was reached in 1,500 minutes without any evidence of the characteristic change of rate which might be expected.

Details of the log-rise, log-time relations are shown in figures 5, 6, 7, and 8. Apparently, the sample conception of an exponential curve giving place to a parabola fails when angular, fine-grained materials are used. There seems to be some evidence that the curve representing the initial arm flattens out approaching a parabola, whereas the second arm, which is perhaps entirely parabolic with spherical material, becomes parabolic with soils only after an appreciable time. Since the curve leading to this parabola is tangent to the modified exponential of the initial phase, all evidence of a discontinuity, such as is noted with sand, disappears. The curves in figures 7 and 8 show no evidence of such discontinuity. However, from soil moisture sampling it is
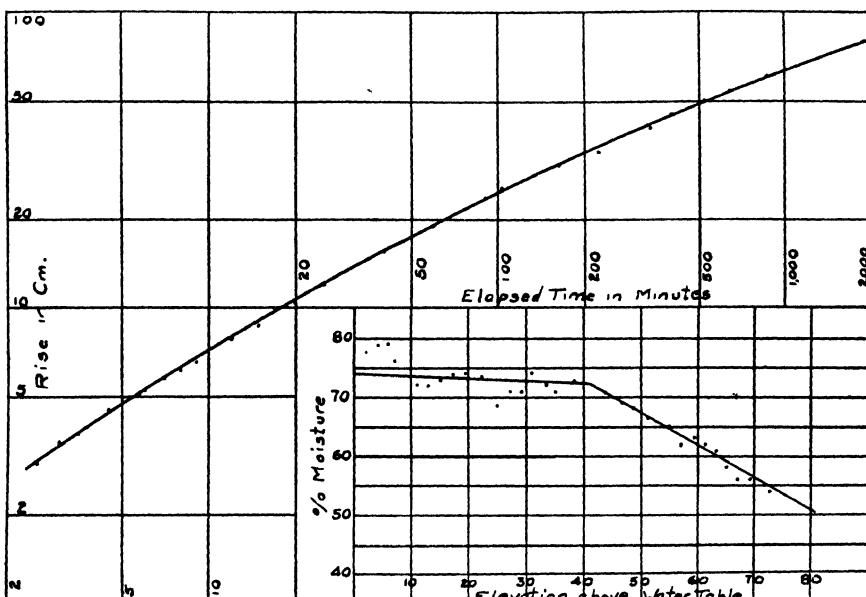
FIG. 8. LOG.-RISE LOG.-TIME CURVE FOR HEATED KUNIA SOIL, TOGETHER WITH RESULTING MOISTURE DISTRIBUTION

evident that some change in the process of rise is introduced at 20 cm. in figure 7 with normal Kunia soil, and at 40 cm. in figure 8. It is assumed that these points mark the ends of the modified exponential rise and the beginnings of the modified parabolic rise. The significance of these points is masked in the log-time, log-rise curves by the continuity of the.curves passing through them.

The distribution of soil moisture in capillary columns

McLaughlin's (7) observation that the distribution of moisture in capillary soil systems is characterized by a zone of high moisture content which extends for some distance above the water table and then by consistently and uniformly

decreasing moisture contents seems to support the assumption of discontinuity of rise proposed above. All efforts to correlate the position of this discontinuity in moisture distribution with the elevation at which exponential rise gives place to parabolic rise with sands, failed because of the difficulty of obtaining a sufficient number of samples from the short column available.

The relation between rates of rise and moisture distribution with emery has already been mentioned and is illustrated in figure 4. Here the column is saturated to an elevation of 19 cm., the rest of the rise being marked by continuously and uniformly decreasing moisture contents. The same elevation marks the beginning of the parabolic aspect of the rise-time curve.

Moisture distribution in the true soils is not so simple. When normal kunia soil (fig. 7) is used, a zone of maximum moisture content, is found at 10 cm., a zone of rapidly decreasing moisture continues to 20 cm., beyond which a more gradual but very consistent decrease continues to 55 cm. Moisture distribution in the heated Kuniz soil (fig. 8) shows no zone of maximum moisture content but a stratum of high moisture content to 40 cm., beyond which fairly uniform decrease occurs. The significant point about such distribution seems to be that a large part of the column is characterized by a uniformly and gradually decreasing moisture content. This section of the column begins when the eratic distribution during the initial phase ceases and continues to the top of the wetted section. Although McLaughlin (7) emphasizes the presence of a zone of maximum moisture content, often encountered in the lower elevations, this seems to be of secondary importance.

Another significant relation between elevation and moisture content is evident from studies with a relatively coarse-grained soil from the Island of Maui. After being air-dried, stored, and screened through a 48-mesh screen, this soil has a moisture equivalent of 28.0 per cent. This mixed material was packed in sectional glass tubes, which permitted intensive sampling within the upper zones of the wetted columns. Three columns were prepared.

The columns were disassembled in the manner already described, when the moisture had reached 66 cm. in one case, 87 cm. in another, and 120 cm., for the third. These elevations may be called W. The fact that the last column had practically reached its ultimate rise was evidenced by the log-time, log-rise curve, which had begun to depart from its parabolic shape and assume an asymptotic value. Moreover, the line of demarkation between wet soil and dry soil became increasingly indistinct at about 114 cm. This characteristic has often been noted as the rise approaches its maximum.

In each case three samples were taken above the significantly wetted column, and enough samples were taken from below this line of demarkation to identify the characteristics of the moisture content-elevation curve. All samples came from the section above the region characterized by a constant slope in the log-time, log-rise curve. When moisture contents below W, in each case, were plotted against elevations, a straight line resulted, whereas moisture contents above W, similarly plotted, fell off rapidly, reaching the air-

dry moisture content in less than 6 cm. In the third case, that in which the rising moisture was allowed to approach its maximum elevation, the discontinuity in the moisture content-elevation curve was not so sharply marked as in the two other cases. The point of change, however, was easily noted at 114 cm. of elevation, which has been reported as the point at which the sharp distinction between the wetted soil and dry soil became less pronounced.

The moisture contents at the elevations of discontinuity in moisture distribution were surprisingly similar, being 32.6 per cent for the 67-cm. column, 32.0 per cent for the 87-cm. column, and 32.0 per cent for the 120-cm. column. The similarity of these values seems significant since they all closely approximate the maximum water-holding capacity for the soil in question. Experience with many Hawaiian soils indicates that the moisture equivalent multiplied by 1.1 gives a fair approximation of the field capacity. The field capacity for the soil used computed in this base was 30.8 per cent. Since the

TABLE 1

Summary of the relation between moisture equivalent and the moisture content at the top of capillary columns

Reported by McLaughlin

SOIL TYPE	NUMBER OF COLUMNS	MOISTURE EQUIPMENT	AVERAGE MOISTURE AT TOP OF WETTED COLUMN
		per cent	*per cent*
Idaho sandy soil	4	4 7	5 5
Riverside soil	1	7 9	9 8
Idaho Lava Ash soil	3	18 3	18 8
Santa Clara soil	3	20 8	22 6
Whittier soil	1	38 3	34 8
Utah soil	1	22 2	20 4

samples came from a 2-cm. section of increasing moisture content, it is evident that the moisture content at the precise top of the wetted section would be somewhat less than that reported.

Moreover, it seems clear that the slope of the moisture-content, elevation curve becomes less as the wetted column increases in length. Apparently this slope decreases to a given value which may be a characteristic of the soil used. Rise by the action of surface forces apparently ceases when this asymptotic slope or "capillary gradient" is reached. The slopes for the three columns listed in the foregoing were 0.293, 0.164, and 0.073 respectively. It is interesting to note that the points obtained by plotting the values of W against the resulting capillary gradient are themselves essentially collinear.

McLaughlin's (7) work on the moisture distribution in capillary tubes shows a similar relation between moisture equivalent and the moisture content at the top of the wetted column, although his results have never been presented

from this point of view. McLaughlin reports studies on six soils with moisture equivalents ranging from 4.7 per cent for an Idaho sandy soil to 38.2 per cent for a Whittier (California) soil. Although the consistent decrease in moisture content in the upper part of his columns is apparent, the termination of this series at the upper end is not so clearly defined as those reported, because in some cases maximum rise had been attained. However, the moisture content at this point can be closely approximated. A summary of results, obtained from an analysis of McLaughlin's work, is presented in table 1.

A typical curve from McLaughlin's work is shown in figure 9. Distribution of moisture in this column is characterized by a zone of maximum moisture content extending to about 8 inches and by uniformly decreasing moisture contents from 8 inches to 20 inches. McLaughlin's zone of maximum moisture contents does not seem to be present in this particular column.
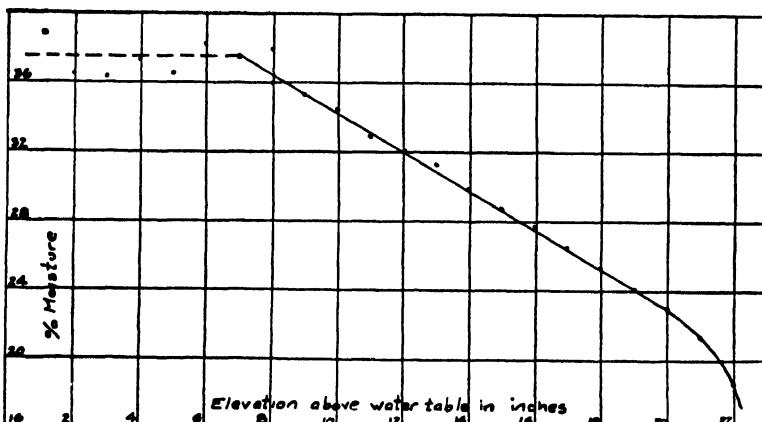
FIG. 9. MOISTURE DISTRIBUTION CURVE FOR SANTA CLARA SOIL (AFTER MCLAUGHLIN)

The effect of the size of container

When the results of observations described in the previous paper (10) are viewed in the light of a modified form of equation (3), the significance of the difference between the performance of large columns and small columns becomes less marked. As has been indicated, the parabolic arm of the rise is common to all such curves, the slopes of these lines, in general, being similar. With soils, however, the second factor of equation (c) $1 - \epsilon^{-ct}$, changes form and has not been determined. This factor apparently influences the time elapsing between zero-hour and the time at which the parabolic rise becomes marked. If so, it may be eliminated from a consideration of the ultimate performance of the column. If this is done

$$R = Kt^n \qquad (J)$$

adequately expresses the relation of R and t, with the provision that t must be relatively large. Table 2 gives the values of n and K for the various column sizes reported, the ratios of perimeter to area, and the elevations of the zones of maximum moisture content for each of the sizes previously reported.

The values of K for each of the sizes are essentially the same, whereas n, the other variable, increases with apparent significance in only the two smallest columns. Columns of the 3-inch, 4-inch, and 5-inch size seem to show increasing ultimate rise because of fortuitous combinations of K and n.

The cause of the small value of n for the smaller sizes is not clear. The longer perimeter per unit cross section may be responsible. More compact packing is possible in internal areas than along the perimeter. If most of the rise takes place in internal elements of the column, it is clear that the rise must be at a slower rate in small columns than in large ones since with the small columns a significantly large part of the water lifted per unit time is lost later-

TABLE 2

Constants for equation (J) together with elevation of zone of maximum moisture content for previously reported columns

ONE DIMENSION OF SQUARE COLUMN	$\dfrac{P}{A}$	K	n	ELEVATION TO MAXIMUM WATER ZONE
inches				*cm*
1	4 0	64 5	0 063	10
2	2 0	60 0	0 134	14
3	1 3	60 0	0 151	26
4	1 0	68 0	0 129	32
5	0 8	65 0	0 141	29
6	0 7	66 5	0 146	33
8	0 5	66 0	0 141	30 5
12	0 3	66 0	0 143	30 5

ally to increase the moisture content at the perimeter, less being available for vertical rise. When larger columns are considered, this effect decreases and apparently becomes insignificant with columns larger than 2 inches square. Subsequent observations with the same soil in cylindrical celluloid columns with diameters of 2 inches and 4 inches showed no difference in rate of rise.

The influence of perimeter is again noted in the position of the zone of maximum moisture content. Significant increases of elevation are noted as the size increases to 2 and possibly 3 inches. Further increase in size is not effective in increasing this elevation.

DISCUSSION

Although much has been written with respect to the physics of capillary distribution from a free water table, the mechanics of the process is still far from clear. Briggs (2) and Buckingham (3) attribute the rise to differences

in the surface curvature of soil water masses lying between adjacent grains. The resulting upward tension is supposed to lift water from the reservoir at the bottom of the column, carry it through the connecting films, and use part of it at least in increasing the radius of curvature of the water surface immediately above the last wetted grain. Because of the ever increasing weight of supported water, the moisture masses between the grains decrease in volume and the enclosing films become thinner as elevation is gained, the moisture percentage supposedly decreasing in the same sense. Moisture presumably ceases to rise when the wedge-shaped masses become insignificantly small.

McLaughlin's (7) observation that such moisture distribution follows no such simple rule, but is characterized by a zone of maximum moisture content appreciably above the water table, has never been satisfactorily explained. Nor can these well-substantiated observations be justified by Briggs' (2) conception.

Keen (5) visualizes capillary rise through soils as being more analogous to the usual phenomena in a capillary tube, assuming, as has Meinzer (8), that the pore space in a soil may be compared to a bundle of capillary tubes of various diameters this variance depending upon the range and distribution of grain sizes involved.

From theoretical reasoning concerning moisture distribution, Keen concludes that we might expect to find three rather distinct moisture horizons in such a capillary system. The lowest of these should, according to Keen, be marked by complete saturation, the next by complete saturation of the smaller pores and incomplete saturation of the larger ones, and finally a region of incomplete and decreasing moisture. Keen's conclusions seem to be well supported by the moisture determinations reported in the foregoing if the region of relatively low moisture content, which is sometimes found immediately above the water table, be disregarded.

As has been indicated, the exponential form of the rise-time curve during the first phase of rise is observed with relatively coarse spherical sand grains as shown in figure 3. This relation is no longer evident when fine, angular materials such as emery or soils are used, although in such cases there is some suggestion of discontinuity in the entire curve. The cause for this change is not clear. Resistance to flow through the irregular capillaries of a mass consisting of irregular fragments probably increases rapidly as size-range and angularity increase. Consequently, the locus of points resulting from plotting rise against time for such materials would fall consistently below the exponential curve, approaching the parabolic form noted in figure 6. Some such form as

$$R = ct^m (1 - \epsilon^{lt}) \qquad\qquad (K)$$

might express the initial phase of rise if the ratio of c to l were considered as a function of the range of grain sizes and their angularity, this ratio being large with such material as inert sand and small for emery. No attempt has

been made to evaluate the constants suggested in equation (K). The colloidal material in the soil with its volume-increase upon wetting adds another factor which would necessarily enter equation (K) if used for other than inert materials. Consequently, equation (K) is of only speculative interest.

Bouyoucos (1) seems to believe that colloidal swelling with the consequent closure of capillary conduits is of dominating importance when soils are under observation. He reports that if columns of dry clay are placed over water and kerosene, the organic liquid at the end of three days will have risen four or five times as high as the water in spite of its lower surface tension, because of the inability of the colloidal material to absorb the kerosene and consequently reduce the effective pore space. Local results fail to substantiate this finding. A Waipio (Oahu) soil of high colloidal content exhibited less rise after 30,000 minutes when supported over kerosene than when supported over water. Moreover, the parabolic curves for the second phase were essentially parallel. Local soil colloids are lean in silica and rich in iron and alumina. These colloids have never been studied with respect to swelling. The low sesquioxide ratio of the Waipio colloid may be associated with a small capacity for volume increase.

Although the relation between moisture distribution and the transition to parabolic rise is marked with emery, the relation is poor with soils. In figure 7 observations upon rate of rise of moisture with an unheated Kunia soil show no parabolic relation between rise and elapsed time until an elevation of 65 cm. has been reached. Moisture determinations, however, indicate a uniformly decreasing moisture content beginning at 20 cm. The possibility of this being due to colloidal activity and a throttling action, brought about in some way by colloidal volume-increase, is discounted by the fact that the same material when heated to 1100°C in an electric muffle, a temperature which according to Whitney (9) should inactivate the colloidal material, showed a similar relation but to a more marked degree. In figure 8 the parabolic relation between rise and elapsed time had not begun at 80 cm., although the discontinuity in moisture content was marked at about 40 cm.

Perhaps a better hypothesis than one involving the conception of swelling colloids, lies in assuming that the true parabolic nature of the second phase of rise is only apparent when soil grains are so large that turbulence with consequent energy loss at the beginning of this phase is negligible. For other materials the second phase, if it is assumed to begin at the point of moisture discontinuity, is characterized by a smooth curve tangent to that of the first phase and becoming parabolic after an elapse of time, which increases with the fineness of grain.

If this assumption is made, the discontinuity of moisture content should be taken as the point at which the rate of soil-moisture decrease with elevation, becomes essentially uniform to the top of the wetted section. This point would be marked by 20 cm. for figure 7, 40 cm. for figure 8, and 19 cm. for figure 4. Irregularities in moisture, lower than this point, as in figure 7 and

as emphasized by McLaughlin (7), probably depend upon a trapping of air in the lower horizons due to rapid rise through the more open passages in the case of increasing moisture, and to the successive failure of certain zones to attain saturation because of large pore spaces caused by varying grain sizes and random packing in the case of decreasing moisture.

As has been indicated, Briggs (2) assumes that capillary equilibrium is attained when the films of moisture surrounding soil grains and the water in the wedge-shaped masses lying between them are insignificant and the moisture content is consequently small. Although such may be the case with inert materials, the analysis of moisture distribution indicates that a soil may contain significant quantities of water under certain conditions and lose it only slowly, if at all, by true capillary movement. The quantitative relation of this water to the maximum water-holding capacity adds interest to Buoyoucos' (1) assumption that a large part of the water in a soil at the maximum field capacity is colloidally imbibed. Whitney (11) seems to hold the same view.

Furthermore, it seems clear from other studies that when a soil is wetted to a moisture content no greater than the maximum water-holding capacity, water is available for distribution to adjacent dry soil at an extremely slow rate. Veihmeyer (9) has demonstrated that dry soils packed in close contact with damp soils, wetted either artificially or by irrigation, have little capacity to increase in moisture content. The same principle, under field conditions, has been shown by many workers in modern studies of the effectiveness of a soil mulch. Conrad and Veihmeyer (4) demonstrate the same principle from a different point of view.

In field studies, in the absence of a free water table, the soil when in equilibrium with gravity is rarely at a higher moisture content than its field capacity. At this moisture content capillary movement, upon the principle outlined in the foregoing, is not a significant factor in moisture distribution. The necessary conclusion is that little or none of the moisture in a normal, well-drained soil is in the films and wedge-shaped masses surrounding the granules comprising the capillary system. If this water is colloidally imbibed, studies in the relation of plants to soil moisture assume a different aspect.

SUMMARY

Some evidence is furnished that capillary rise of water through inert sands and through soils is a compound process dominated in the first phase by an action which may result in a complete filling of the pore spaces, and in the latter phase by an action which results in a continuous, uniform decrease in moisture content.

When coarse, inert materials are used, the transition between these two processes can be noted by inspection of the log. rise-log. time curve. With fine materials inspection of the moisture distribution is required.

The minimum moisture content reached by true capillary distribution in soils is about the maximum field capacity. Subsequent movement is at a very slow rate and must be attributed to other causes.

It seems clear that soil-moisture under normal field conditions cannot possess much motility since such moisture contents are rarely more than the maximum water-holding capacities of the soil in question.

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EFFECT OF REPLACEABLE SODIUM ON SOIL PERMEABILITY[1]

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Experience has shown that a good soil contains a large amount of replaceable bases. Such a soil can function more efficiently as a pH regulator. The ultimate state of dispersion, however, depends not only upon the amount of the replaceable bases but upon their relation one to another. From a practical standpoint it is known that calcium in the exchange complex acts favorably upon the soil structure, whereas sodium has an opposite effect, leaving the soil sticky and impermeable.

The reclamation of alkali lands depends to a great extent upon soil permeability, determined largely by its degree of dispersion. In this study of the effect of replaceable sodium in various amounts on soil permeability, it was hoped to obtain a mathematical expression for permeability in terms of replaceable sodium or in terms of the ratio of sodium to the total replaceable bases. The value of such an expression is evident. It would enable the investigator to compare approximate speed of water penetration for different relative amounts of sodium saturation and thus be a satisfactory guide as to the type of reclamation to be pursued. When the relation between soil permeability and replaceable sodium is known it is also possible to calculate the time required to replace sodium by calcium in a calcareous soil by continuous leaching with irrigation water. The calculation given is theoretical in part although based largely upon the experimental relation between (k)[3] transmission constant and (S) ratio of replaceable sodium to total replaceable bases.

EXPERIMENTAL

The experimental work was conducted on a heavy, calcareous, alkali soil of Cache Valley. The samples collected from the surface horizon varied widely

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Note: There is no pressure head, but a constant force of 980 dynes is acting on each gram.

TABLE 1

Analyses of replaceable bases

LABORA- TORY NUMBER	DEPTH	CO_2	DISSOLVED ANIONS* Percentage		REPLACEABLE BASES MILLIEQUIVALENT PER 100 GM						TOTAL BASES	(NH_4) ABSORBED	RELATIVE PROPORTIONS				$T \times 10^{+4}$
					Total		Corrected										
			SiO_4	CO_2	Ca	Mg	Ca	Mg	Na	K			Ca	Mg	Na	K	
	inches	*per cent*															
1878	6	6 87	0 265	0 38	30 5	11 1	9 5	6 1	3 4	1 1	20 1	15 6	49 6	32 0	17 9	0 5	4 1
1879	15	17 8	0 036	0 42	23 0	11 0	7 0	7 0	2 7	1 1	17 8	13 8	39 2	39 2	15 2	6 4	4 5
1882	60	14 6	0 144	0 32	21 0	11 0	5 7	7 0	6 2	2 3	21 2	14 9	26 9	35 0	29 3	10 8	0 5
1872	4	0 34	0 203	0 125	18 4	8 5	8 5	6 0	2 7	0 04	17 2	14 3	49 4	34 8	15 7	0 1	6 2
1873	14	9 60	0 182	0 330	20 8	10 5	4 0	6 2	9 7	0 1	19 0	14 8	21 0	32 6	51 0	0 4	1 2
1876	50	18 1	0 077	0 250	12 7	9 2	1 7	6 4	7 3	0 44	15 9	11 7	10 7	40 2	45 9	3 2	1 3
1867	8	1 24	0 154	0 210	14 0	3 0	2 2	0 2	18 0	0 2	20 6	17 5	10 6	0 95	87 5	0 95	0 38
1868	18	14 2	0 075	0 410	16 1	8 5		3 4	14 2	0 1	17 7	17 2		19 2	80 1	0 7	0 80
1871	60	11 4	0 210	0 220	14 9	6 4	0 9	3 4	13 2	0 3	17 8	15 0	5 0	19 1	74 4	1 5	0 20
1884	8	2 76	0 101	0 260	15 5	7 1	3 4	4 1	10 4	0 7	18 6	17 4	18 3	22 0	56 0	3 7	0 94
1885	20	20 8	0 062	0 300	15 0	10 0	3 0	6 4	9 2	0 7	19 3	16 2	15 5	33 2	47 8	3 5	1 2
1887	50	18 2	0 110	0 240	12 4	11 5	1 4	7 8	5 8	1 2	16 2	12 5	8 6	48 0	35 8	7 6	2 0
1888	6	1 58	0 140	0 162	16 0	8 5	6 4	6 1	7 2	1 2	20 9	18 7	30 6	29 2	34 4	5 0	0 5
1889	14	20 5	0 043	0 410	14 4	10 0	6 4	4 4	11 0	1 5	16 9	13 5		26 0	65 0	9 0	0 6

* Anions dissolved from KCl treatment

in replaceable sodium, and a minor variation was obtained in the second calcareous horizon. Sampling was made from a vertical face, making it possible to obtain a representative sample of a definite horizon; it is felt that the samples thus collected from a small alkali tract differ mainly in the extent of

TABLE 2

Concentration of anions and cations leached from soils one year after percolation had begun.
(Expressed in milliequivalents per 100 cc. of solution)

LABORATORY NUMBER	SiO$_2$	Cl	SO$_4$	CO$_2$	HCO$_3$	Mg	Ca	K	Na	TOTAL ANIONS	TOTAL CATIONS
1767	0 046	0 020	0 002	0.240	0 775	0.218	0 040	0 175	0.786	1 08	1.22
1798	0.043	0.016		0 056	0 397	0.230	0 223	0.031	0.117	0.512	0 60
1868	0 066	0.044	0.088	0 450	0.935	0.434	0.067	0.200	1 03	1.58	1.73
1878	0 054	0.020		0 080	0.536	0 375	0.252	0.032	0 208	0.29	0 85
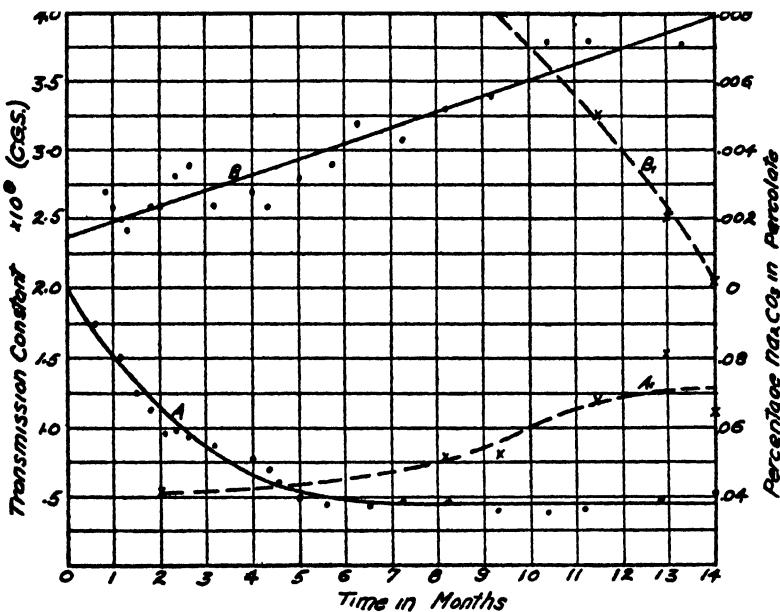
FIG. 1. *A* AND *B* TYPICAL TIME CURVES FOR THE RATE OF PERCOLATION (READ LEFT ORDINATE)

A_1 and B_1 alkalinity of percolate for the same time interval computed as percentage Na$_2$CO$_3$ (read right ordinate). Note: Two scales.

chemical weathering or the extent to which sodium in the soil solution had replaced calcium and magnesium from the exchange complex.

Chemical analyses were made on all samples for soluble salts, total carbonate, and replaceable bases (table 1). Percolating cans 12 cm. in diameter and 38 cm. long with a capacity of 4 kgm. of air-dry soil, were set up in triplicate, the height of water at intake and outlet so arranged as to give no pressure head.

Representative time curves for the rate of percolation of water containing approximately 35 p.p.m. of Ca through soil columns with different amounts of replaceable sodium are shown in figure 1. The downward trend in curve *A* is due to the decrease in soluble salts and subsequent hydrolysis of sodium from the colloids, producing marked alkalinity and deflocculation. This curve represents soil 1867, which contains 1 per cent soluble salts, largely sodium chloride, and is 87 per cent sodium saturated. After two months' leaching, the percentage of sodium chloride in the extract computed from the chlorine titration was 0.096. After 10 months this value was reduced to 0.024, accompanied by an increase in the alkalinity of the percolate (curve A_1). The experimental curves (A and A_1) for the limited time, seem to indicate that the lowest value of k has been reached, or in other words, the final state of dispersion has been obtained. A similar analysis for all the soils makes it possible to identify a characteristic transmission constant with a definite sodium saturation. A different trend is obtained for soils that are low in replaceable sodium (curve B). This sample was considerably lower in soluble salts (0.22 per cent), and 18 per cent sodium-saturated. The gradual increase in the transmission constant is associated with the marked reduction in the alkalinity of the percolate (curve B). Since a larger percolation rate maintains (nine times as fast as in the former case), the result will be a rapid disappearance of replaceable sodium from the system. It is to be noted that the percentage of sodium carbonate is approaching a zero concentration on the fourteenth month (see upper scale on right ordinate), but the percolate is well on the alkaline side of neutrality; thus, there is a reason to believe that the soil will continue to improve with leaching, k getting larger until a maximum value is obtained.

The percolation studies were made in triplicate columns and the conductance of the percolate, together with the transmission constant, was observed over a long period of time. Any appreciable variation in the rate due to channels developing in the soil mass can be detected by the conductance measurement.

METHOD OF DETERMINING REPLACEABLE BASES

In view of the uncertainty of determining accurately replaceable bases in calcareous soils, it seems necessary to explain briefly the method used. The common ammonium chloride method will not suffice for calcareous soils because of the solubility effects of silicates and carbonates of calcium and magnesium. These difficulties, accompanied with a high concentration of soluble salts whose removal affects the exchangeable constituents, make it reasonable to expect inaccuracies in any method. In brief, the following procedure was adopted:

Replaceable sodium and potassium were determined from an NH_4Cl treatment, since their determination and replacement are not influenced by solubility effects. However, due allowance is made for the water-soluble cations in the soil previous to treatment.

Replaceable calcium and magnesium were determined from a KCl treatment, but correct-

ing for the Ca and Mg equivalent to the loss in carbonates plus dissolved silica in the solution, assuming in the latter case a solution of a metasilicate of calcium and magnesium.

The NH₄ absorbed was determined by the usual method as a reasonable check on the total bases.

It is to be noted in table 1 that the correction for dissolved anions is distributed between the total Ca and Mg determinations in a definite ratio. For example, three successive comparable treatments after the replaceable divalent cations were determined gave a constant ratio of Ca to Mg in the solution, namely 4 m.e. of Ca to 1 of Mg. Therefore, by expressing dissolved CO_2 and SiO_2 in m.e. per 100 gm. and dividing the sum in the ratio of 4 to 1, the correction can be made from total Ca and Mg determination and the relative amounts of the replaceable divalent cations can be obtained. It should be pointed out that this is only a suggestive possibility for distributing the solubility effect and has no bearing on the experimental relations here considered. For in this work an accurate measure of the total bases was desired so that the ratio of replaceable sodium to the total could be obtained with the same degree of accuracy.

In connection with the KCl treatment, it is to be noted that the solution of carbonates and silicates is greater in an NH₄Cl treatment than in a KCl treatment. Thomas (3) reports an approximate threefold increase in dissolved silicates, and a fivefold increase in dissolved carbonates where NH₄Cl was used as the displacing agent, as compared with KCl or NaCl. Under these conditions a KCl treatment will give a more complete replacement of Ca and Mg. This is illustrated in table 1. The total amount of bases determined exceeds the amount of NH₄ absorbed by consistent amounts, except where the soil is practically Na saturated, and here the agreement is better. This shows that in the absence of replaceable Ca and Mg equivalent absorption and replacement of Na and K can be analytically realized.

The constant presence of dissolved calcium during the NH₄Cl treatment not only prevents the replacing action from going to completion, but it is likely that Ca ion is acting as the displacing cation instead of NH₄. This point is emphasized because sample 1876 gave 18.2 m.e. of Na and K replaced with a less equivalent absorption of ammonia.

DISCUSSION OF RESULTS

From the experimental data in figure 2 it would seem that a logarithmic relation between k and S will approximate the facts. The permeability obtained from varying a single factor (S) can be expressed in curves which are asymptotic to a minimum. The empirical relation is obtained by the ordinary method of plotting $ln\ k$ against S and evaluating the constants. The extreme high and low values of S are extrapolated, but the experimental range from S equals 12 to 87 includes the major part of the curve. Comparing the two relations obtained from the surface and $CaCO_3$ horizons, one is led to conclude that the calca-eous horizon when free from alkali, or in the best

physical condition, is more impervious than the surface 18 inches. On the other hand, being a more compact soil in an alkali-free condition, the latter horizon will respond more slowly to corresponding increments in S resulting in a higher percolation rate at saturation. In this connection it may be noted that Greenville soil is very loamy and permeable ($k = 30 \times 10^{-9}$) in the normal Na-free conditon, but when treated with NaCl and washed free from salts the value of k decreases to 0.3×10^{-9}. This is the same order of magnitude obtained in the foregoing for a heavier clay soil. In this case only the two extreme values can be given and not the complete curve.
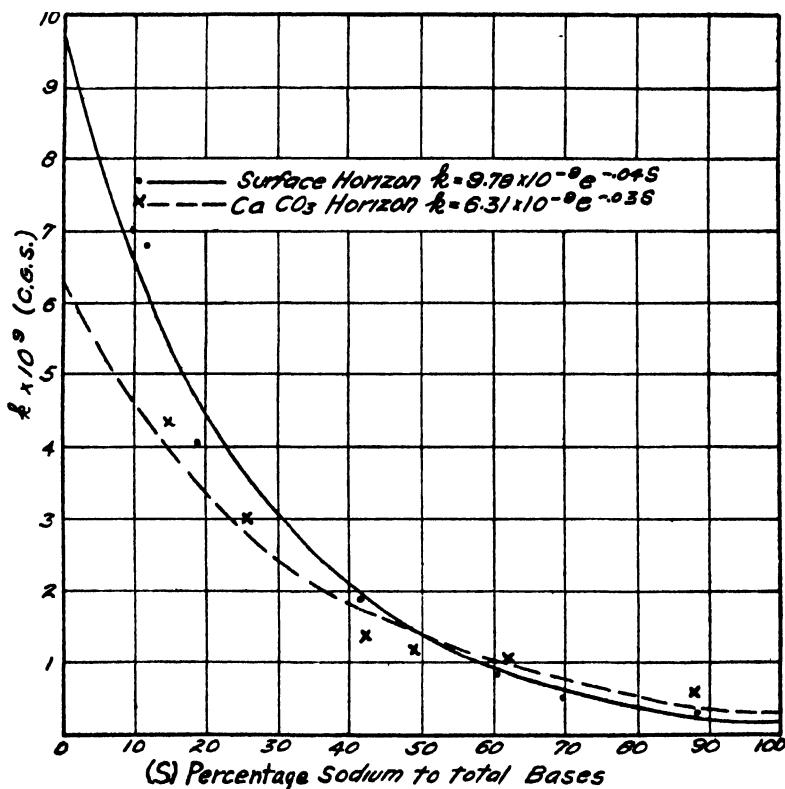
FIG. 2. RELATION BETWEEN TRANSMISSION CONSTANT AND PERCENTAGE OF SODIUM FOR TWO HORIZONS

Reference has been made by Kelley and Brown (2) to the effect that a calcareous soil saturated with replaceable sodium can be reclaimed without chemical treatment by leaching and drainage. Burgess (1) has also shown that the reclamation of calcareous alkali soil by leaching only (without applying gypsum) is possible, but it is an extremely long and tedious process unless irrigation water high in soluble calcium is available. This slowness is due to the extremely low solubility of $CaCO_3$ in alkaline soil solutions. The gradual

change of replaceable sodium into replaceable calcium during leaching has been shown by various investigators, but the length of time required to bring about a quantitative change is our immediate concern. The solution of this problem centers around the setting up of a differential equation containing the necessary variables. Seemingly, a differential equation is necessary since the sodium will disappear from the solid phase and hence be leached out at a variable rate as a result of the changing concentration and percolation rate as time goes on. If the rate at which the replaceable sodium disappears from a homogeneous soil column is proportional to the total replaceable Na present (which would seem to harmonize with the generalized law of mass action) and at the same time is proportional to the permeability of the soil, the following differential equation to express this hypothesis may be used:

$$- \frac{dC}{dt} = KCk \qquad (A)$$

where C = total exchangeable sodium in a given soil column (4 kgm.), k = transmission constant (in C.G.S. units), K = proportionality factor, and t = time (seconds). The importance of permeability as a variable factor cannot be overemphasized, because the degree of hydrolysis and the extent of replacement of sodium by calcium are determined by the rate of dilution. The soluble products (NaOH) from hydrolysis, Na_2CO_3 and $NaHCO_3$ from Ca replacement) must be removed before the reaction will continue with any rapidity.

In order to obtain a solution to equation (A), it is necessary, first, to express k in terms of C, and, second, to determine the proportionality constant by measuring experimentally the derivative for different values of C. This would give some assurance as to the validity of the expression over a wide range. Since C and S are related by the expression (A') $S = \frac{C}{C_t}$ where C_t is equal to the total amount of replaceable bases, and assumed constant for a given soil, it follows that

$$\frac{dS}{dt} C_t = \frac{dC}{dt} \qquad (B)$$

By eliminating C and $\frac{dC}{dt}$ from equation (A) by using equations (A') and (B) the following differential equation[4] is obtained:

$$\frac{dS}{dt} = K_1 Sk; \text{ where } K_1 = - K \qquad (C)$$

By expressing k in terms of S from the experimental relation shown in figure 2, the following equation is obtained:

[4] The negative sign was used in the first place because C decreases and consequently S, as the time increases.

$$\frac{dS}{dt} = K_1 S\, 9.78 \times 10^{-9}\, e^{-.04\, S} \tag{D}$$

or

$$K_1\, 9.78 \times 10^{-9}\, dt = \frac{e^{.04\, S}\, dS}{S} \tag{E}$$

Integrating equation (E) will give the following functional relation between t and S:

$$K_1\, 9\,78 \times 10^{-9}\, t = ln\, S + .04\, S + \frac{04^2\, S^2}{2 \cdot 2!} + \frac{04^3\, S^3}{3 \cdot 3!} + \dots \tag{F}$$

This series converges rapidly because of small numerical values of S, and, since definite limits will be used, the integration constant vanishes. After K is evaluated in equation (F), it remains to substitute any desired limits in

TABLE 3

Values of $\dfrac{dc}{dt}$, C, k, and K^1 for four soils

LABORATORY NUMBER	k	C	$\frac{dc}{dt}$ *	K^1
1767	$1\,2 \times 10^{-9}$	8 16	$2\,31 \times 10^{-8}$	2 36
1868	$0\,77 \times 10^{-9}$	11 70	$2\,00 \times 10^{-8}$	2 21
1878	$4\,10 \times 10^{-9}$	2 22	$1\,97 \times 10^{-6}$	2 16
1798	$6\,4\ \times 10^{-9}$	1 11	$1\,63 \times 10^{-8}$	2 30

* $\dfrac{c^2 - c_1}{Seconds}$ or total grams of replaceable Na that disappeared from a 4-kilogram soil column per second

S and calculate the time in seconds necessary to effect such a reduction in replaceable Na.

The constant K_1 was obtained from the original supposition as given in equation (A) by measuring corresponding values of $\dfrac{dC}{dt}$, C, and k (table 3). When the values obtained for soils containing different amounts of replaceable sodium are compared, it is evident that the proportionality constant varies within narrow limits; this is principally because of the experimental method of measuring $\dfrac{dC}{dt}$. These data constitute an experimental verification of the original assumption. The rate of Na replacement was determined by analyses of the percolate for total anions and cations (table 2). The total Na determination was reduced by the sodium equivalent to the chlorides and sulfates and the remainder was assumed to have come from the soil colloids. This reduction is noticeably small because the analysis was made about a year after percolation had begun. The question still

remains as to whether replaceable Na is disappearing at a finite and measurable rate, of course, realizing that it would be desirable to measure this average rate over a small time interval. Ample Na for a determination can be obtained from one month's leaching, and it is felt that the difference in the values of C at the beginning and end of the month divided by t in seconds, will approximate the derivative with all the accuracy that would be consistent with the previous analysis of the problem. This average rate for the time interval considered can be interpreted for all practical problems as an actual instantaneous rate, especially in view of the fact that very little
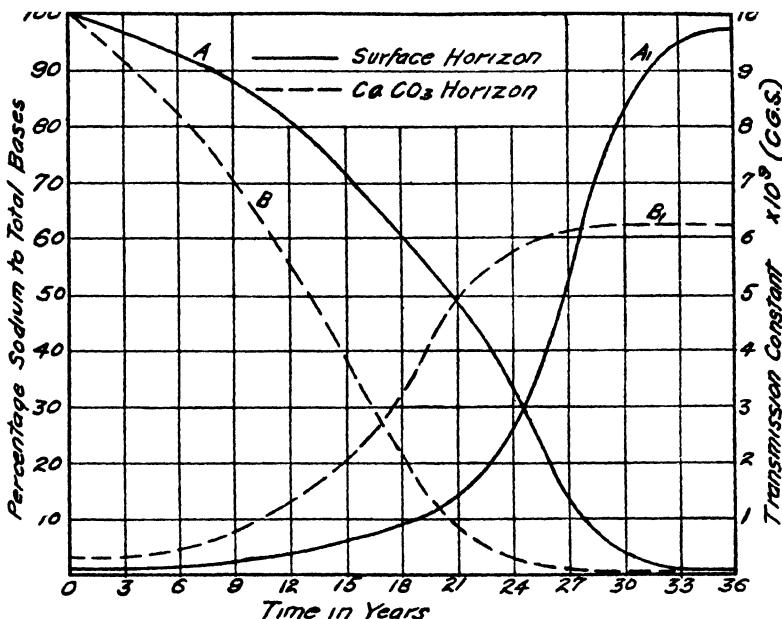
Fig. 3. *A B* Change in Sodium Percentage with the Time (Read Left Ordinate) and A_1 B_1 Shows Increase in the Rate of Percolation with the Time (Read Right Ordinate)

or no measurable change in k would result from a 20 to 50 mgm. reduction in replaceable Na from a soil column containing 6 to 16 gm.

The results of table 2 also emphasize the rôle soluble calcium plays during the leaching. Such large differences in the calcium content of the percolates cannot be due to a difference in the solubility of calcium carbonate. Moreover, the low calcium values from the highly sodium-saturated soils seems to be due to the replacement of Na by Ca and subsequent formation of large quantities of sodium carbonate and sodium bicarbonate. This reaction is going on in the other samples which are low in replaceable Na and where high Ca extracts were obtained, but to a less extent because of the limited amount of replaceable Na present.

A clear interpretation of equation (F) can be obtained from figure 3 (see curve A). A similar equation involving the experimental relation for the $CaCO_3$ horizon, which changes only the constants, gives curve B. This curve has a greater slope for large values of S, which is due to a larger percolation rate at saturation resulting in a greater net amount of soluble products removed. For convenience the ordinate was shifted in order that 100 per cent would correspond to zero time in each case. The corresponding curves (A and B) involving the transmission constant as a function of the time were deduced from equation (F) and the analogous equation for the $CaCO_3$ horizon, by expressing S in terms of k. These curves give a complete time history of the transmission constant until replaceable Na is reduced to a point where its presence or absence from a soil mass has little or no influence upon the transmission constant. It would require more than 20 years to verify these curves experimentally, but it is of interest to note that the experimental curve B in

TABLE 4

Reduction in replaceable Na due to continuous leaching under field conditions

LABORATORY NUMBER	RELATIVE PROPORTIONS IN SURFACE HORIZON					LABORATORY NUMBER	RELATIVE PROPORTIONS IN CaCO₃ HORIZON				
	Ca	Mg	Na	K	AVERAGE PER CENT Na		Ca	Mg	Na	K	AVERAGE PER CENT Na
1893	33 4	15 8	40 0	10 8		1894	33 3	6 0	60 1	0 6	
1897	45 0	16 5	34 0	4 5		1898	16 9	21 7	53 0	8 4	
1903	43 2	32 0	23 0	1 8	32 3	1904	15 7	28 6	49 4	6 7	54 8

Results for comparable samples after 2 years' leaching

1895	54 0	16 7	21 0	8 3		1896	33 7	28 2	38 1	0 6	
1899	49 4	34 6	16 0			1900	32 1	23 3	37 9	8 4	
1901	63 1	21 2	12 3	3 4	16 4	1902	24 4	38 4	31 5	6 7	35 8

figure 1 has approximately the same slope as the theoretical curve A in figure 3 would demand. That is to say, when the transmission constant is in the neighborhood of 3.0×10^{-9}, the rate at which it increases is of the order of magnitude that the calculation gives.

A comparison of the time values here given with reduction in replaceable Na, resulting from leaching conditions in the field, can be obtained from table 4. A few years ago on this alkali tract small pressure wells were drilled into the gravel stratum, with the thought of measuring artesian pressure. After 2 years of continuous flowing, soil samples were taken from the surface and $CaCO_3$ horizons in the immediate vicinity of the wells. The analysis for replaceable bases on the leached and unleached soils is shown in table 4. The results from three different locations are averaged in each case because sampling was more or less at random. As a result of 2 years' leaching with artesian water, evidently replaceable Na has been reduced in the surface horizon from 32.3 to 16.4 per cent and in the $CaCO_3$ horizon from 54.8 to 35.8 per cent.

Referring to the former curves, figure 3, one obtains approximately 2.3 and 2.7 years, respectively, for corresponding reductions. Obviously, field and laboratory conditions are not closely comparable. Under field conditions there are at least four factors influencing the results and which do not exist under laboratory conditions:

The liberation of CO_2 by decaying organic matter, furnishing a better solvent for the solution of $CaCO_3$.

Surface run-off carrying injurious alkalinity.

Higher Ca content in the water used, which was especially true in this case

Fresh water is not applied to the lower horizon. The leachings from the surface soil will contain Na ions which will tend to retard the ionization of Na zeolites. However, this retarding effect, in part or wholly, may be compensated for by the first factor.

These factors, together with the experimental results, seem to point toward a faster reclamation under field conditions, which is more encouraging.

From the data here presented and discussed it is evident that the relation between replaceable sodium and soil permeability harmonizes with the contention held by various investigators, namely, that as Na is substituted for the normally occurring calcium in the exchange complex the soil becomes less and less permeable to water. Also, if the substitution goes very far marked alkalinity will be produced. There is no critical danger point, but the effect is highly pronounced at 30 per cent saturation, increasing at a slower rate thereafter. The decrease in permeability soon begins to manifest itself in highly Na-saturated soils, as was pointed out in figure 1, curve A. No single case was encountered where water penetration ceased altogether, even in soil samples taken from slick spots which most writers would pronounce as 100 per cent Na saturated. Although this may be the case in non-calcareous soils, as various investigators have shown, it is felt that for calcareous soils a finite and measurable rate of percolation maintains. This argument may be advanced in favor of the reclamation of calcareous alkali soils by flooding and drainage, whereas a non-calcareous soil would require the application of a chemical treatment.

SUMMARY

Studies on calcareous soils to determine a relation between soil permeability and replaceable Na show that the permeability decreases exponentially as the Na content increases.

When free from alkali that soil higher in humus is more pervious than the calcareous horizon. The reverse is true, however, when the soils are Na saturated.

The effect of long-time leaching on replaceable sodium and soil permeability is deduced. The time values given in figure 3 serve as a guiding theory for the reclamation of calcareous alkali soils. For the surface horizon and leaching with water containing approximately 35 p.p.m. of calcium, one obtains 18.8 years as the time required to reduce replaceable sodium from 88 to 10

per cent.   A corresponding reduction for the calcium carbonate horizon could be realized in 15.6 years.   Seven years would be required to obtain a reduction from 50 to 10 per cent.

Data are presented to show the reduction in replaceable Na as a result of continuous leaching under field condition.   The results indicate that a faster rate of reclamation is possible under field conditions than is indicated by the laboratory work.   The data, however, are not voluminous enough to obtain a factor by which the rates given in figure 3 could be discounted and obtain a value that would be comparable to field conditions.

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REPLACEABLE IRON AND ALUMINUM IN SOILS

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The researches of Magistad (8), Joffe and McLean (5, 6), and others have shown that the presence and amount of soluble iron and aluminum in soils are dependent upon the hydrogen-ion concentration of the soil solution and upon the nature, and possibly also the concentration, of the anions present. Our knowledge of the factors that determine the soil content of replaceable iron and aluminum, however, is less well defined. For example, it is known that iron and aluminum do not always appear in the filtrate when acid soils are extracted with a neutral salt solution, but the circumstances which determine their presence or absence are somewhat obscure. Further, although Mattson (9) has demonstrated that iron and aluminum may continue to be replaced from the soil material as often as it is rendered unsaturated, yet the relationship which the quantity of trivalent ions bears to the state of unsaturation of the soil remains uncertain. In addition, doubt exists as to whether replacement is direct, through exchange with the cations of the neutral salt used, or indirect through interaction with released hydrogen ions.

For these reasons a study has been made of the influence exerted by certain factors upon the soil content of these replaceable bases. The following soil factors have been investigated: pH value and content of replaceable hydrogen.

SOILS EXAMINED

The soils examined belong to two distinct types: soil type B—a fawn-colored river alluvium; soil type C—a red-weathering clay. The pH values and contents of replaceable hydrogen of the soils of both types cover a wide range.

Some of the physical and chemical constants of the types are recorded in table 1. The determinations were made on samples obtained by bulking the individual soils for which data are given in table 2. Organic matter was measured by the method described by Hardy (3), and clay by the pipette procedure adopted in 1928 by a Sub-Committee of the Agricultural Education Association (13).

Additional information concerning the types may be obtained elsewhere (14).

Soil types B and C are predominantly siliceous. Both are deficient in organic matter. Their saturation capacities, clay contents, and moisture contents at the point of stickiness indicate that both types are highly colloidal.

Examination of the individual soils has shown that each type is fairly uniform in character. The extent to which the various samples differ in their content of organic matter and clay does not appear large enough to exert an appreciable disturbing effect upon the amounts of replaceable iron and aluminum which they may contain.

EXPERIMENTAL

The pH values of the soils and soil extracts were determined by means of the quinhydrone electrode. In the former case a ratio of soil to water of 1 to 2 was used.

Replaceable iron and aluminum were measured on 50-gm. representative samples of the air-dried, soils, which were treated with hot normal sodium chloride solution, stirred, allowed to stand overnight, decanted several times, transferred to funnels, and leached.

Joffe and McLean (6) have shown that iron and aluminum may continue to be replaced from soils, by repeated extraction with normal barium chloride solution, until the filtrate attains a pH value of 5.4 to 5.6. After 1 liter of

TABLE 1

Some constants of the soil types examined

SOIL TYPE	$\frac{R_2O_3}{SiO_2}$	$\frac{Fe_2O_3}{Al_2O_3}$	LOSS ON IGNITION	ORGANIC MATTER	CLAY	SATURATION CAPACITY	M P S
			per cent	*per cent*	*per cent*	*m e* *	*per cent*
B	0 58	0 73	13 58	1 07	68 9	24 2	46 1
C	0 43	0 44	11 76	1 28	50 6	24 3	39 0

M.e. = milligram equivalents per 100 gm. soil.

extract had been obtained, small samples of the filtrate still leaching through were therefore examined for the presence of trivalent ions. Aluminum was present in a number of cases, but iron was invariably absent. The occurrence of aluminum in the filtrate samples was shown to be dependent on their pH value. Aluminum ceased to be displaced by the first liter of sodium chloride solution only when the filtrate samples had attained a pH value of 6.5 to 6.6, a hydrogen-ion concentration approximately one-tenth as great as that recorded by Joffe and McLean (6). The discrepancy is probably due to the difference in the nature of the replacing cation used, and possibly also to a difference in concentration of the chloride ion in the leaching solutions.

The soils which still contained aluminum replaceable by this process were transferred to beakers, and again decanted and leached with normal salt solution. Replacement was found to cease when an additional half liter of filtrate had been obtained, except in the case of the most highly acid and unsaturated soils. The amount of aluminum present in the third half-liter in some cases exceeded 0.7 m.e. per 100 gm. of air-dry soil.

The iron and aluminum present in each filtrate were separately determined,

due precautions being taken to remove any manganese and other metals, and any silica and phosphate present.[1] The trivalent bases were also weighed together as ignited oxides. The data obtained by the two methods showed close agreement.

Replaceable hydrogen was determined indirectly on 25-gm. samples of the soils. Exchangeable calcium was measured by the method of Hissink, and saturation capacity by leaching to 2 liters according to a method based on that

TABLE 2

pH value, content of replaceable iron, aluminium, and hydrogen; and thiocyanate color of soils examined

		SOIL TYPE B						SOIL TYPE C			
Soil number	pH value	Replaceable			KCNS color*	Soil number	pH value	Replaceable			KCNS color*
		Fe	Al	H				Fe	Al	H	
		m e	*m e*	*m e*				*m e*	*m e*	*m e*	
M 48	6 55	Trace	0 14	8 25	0	M101	7 51	0 00	0 00	5 46	0
M 28	6 50	0 00	0 00	6 25	0	M 64	7 14	0 00	0 00	4 21	0
M 46	6 42	0 00	0 00	4 54	0	M 96	6 87	0 00	0 00	3 64	0
M 27	5 79	Trace	0 11	9 25	2	M163	6 74	0 00	0 00	4 50	0
M 22	5 63	Trace	0 22	9 00	5	M157	6 67	0 16	0 11	12 11	
M 24	5 31	0 00	0 00	7 54	23	M100	6 61	0 00	0 00	4 89	0
M 25	4 93	Trace	0 33	12 50	45	M 71	6 07	0 00	0 00	6 54	0
M 44	4 92	Trace	0 56	14 61	25	M 67	5 87	Trace	0 33	8 46	
M 49	4 75	0 10	0 50	13 96	36	M160	5 60	Trace	0 11	11 61	7 5
M 45	4 65	0 15	2 67	17 32	50	M123	5 50	Trace	0 33	8 25	
M 30	4 58	Trace	2 48	15 96	62	M155	5 08	0 00	0 22	15 29	30
M 29	4 53	Trace	0 33	12 86	46	M158	5 08	0 16	0 56	16 64	36
M 23	4 53	Trace	4 83	17 50	60	M164	4 98	0 16	0 39	9 86	31
M180	4 38	0 16	2 26	16 61		M161	4 95	0 04	0 22	14 54	35
. .	. .		.		.	M165	4 77	Trace	2 18	17 32	41
						M159	4 55	0 16	3 44	19 68	64
. . .	. .		.			M156	4 48	0 00	5 20	20 21	50

* Based on an arbitrary unit.

of Page and Williams (14). An estimate of the soil content of replaceable hydrogen, or saturation deficit, was obtained by difference.

The data thus obtained are recorded in table 2.

DISCUSSION AND CONCLUSIONS

Replaceable iron

Mere traces of iron were replaced from the majority of soils examined. Measurable amounts, which in no case exceeded 0.2 m.e. per 100 gm. of soil,

[1] The procedure followed was devised by Mr. H. H. Croucher, formerly of the Imperial College of Tropical Agriculture, Trinidad, to whom the author is indebted for the details of the method.

were found more frequently in the red-weathering clays than in the alluvial soils. The occurrence of replaceable iron in such meager quantity is in common with the experience of other workers (2, 6).

In the case of the soils of type B, iron first becomes replaceable in determinable amount at a pH value of 4.75 and at a saturation deficit of 14 m.e. With type C the corresponding pH and deficit values are 6.67 and 10 m.e., respectively. Replaceable iron was found to be absent, or present in traces only, however, in many soils with lower pH values and higher saturation deficits. In consequence, no well-defined relationship is evident between the quantities of iron present in the filtrates and either of these soil factors.

Replaceable aluminum

The maximum amount of aluminum, replaceable by the treatment described, in the soils of types B and C is 5.2 m.e. In other Trinidad soils as much as 30.7 m.e. have been found. Smolik (12) has shown 13.5 m.e. to occur in soils of the podzol type, Joffe and McLean (6) 14.5 m.e. in New Jersey soils, and Conrey and Schollenberger (2) 2.1 m.e. in a Clermont silt loam subsoil.

The large amounts of aluminum, relative to those of iron, which are replaceable from the soils of types B and C may be of considerable importance in determining the nature of the end products of the weathering processes to which these soils are subject.

Theoretically, the sum of the equivalents of the replaced iron and aluminum should be considered in relation to the pH values and saturation deficits of the soils examined. As the amounts of iron contribute little or nothing to the sum totals of the trivalent bases, they have been neglected in the main discussion which follows.

pH value

The hydrogen-ion concentrations of the soils are plotted against their contents of replaceable aluminum in figure 1.

Although there is a general tendency for the amount of aluminum to increase with increasing hydrogen-ion concentration, the points do not fall even approximately upon a smooth curve. Aluminum is invariably absent from the filtrates obtained from the soils with pH values between 7.5 and 6.7, but always appears when the pH value of the soil lies below 5.1. It may or may not be present over the pH range 6.7 to 5.1. At pH values greater than 4.8 the quantity of replaceable aluminum does not exceed 0.6 m.e. At pH values smaller than this it may rise rapidly, but irregularly, in amount.

The hydrogen-ion concentration of the soil, therefore, does not appear to be the only factor of consequence which controls the presence and amount of replaceable aluminum. A similar conclusion can be drawn from the data recorded by Conrey and Schollenberger (2) which are included in figure 1 for the purpose of comparison.

The consistent appearance of replaceable aluminum at pH values below 5.1 in the soils of both series is of interest, for, according to Pryanishinikov and Lukovnikov (10), the aluminum ion is not adsorbed by soils with pH values greater than 5.0. It is possible, therefore, that aluminum becomes directly exchangeable in the region of this hydrogen-ion concentration.

This view is to some extent confirmed by the fact that, without exception, the soils of pH value less than 5.1, *whether their contents of replaceable aluminum were large or small*, alone needed leaching to more than 1 liter to ensure complete replacement. Slow replacement is to be expected if aluminum is present in the exchangeable form, for the trivalent aluminum ion, because of its rela-
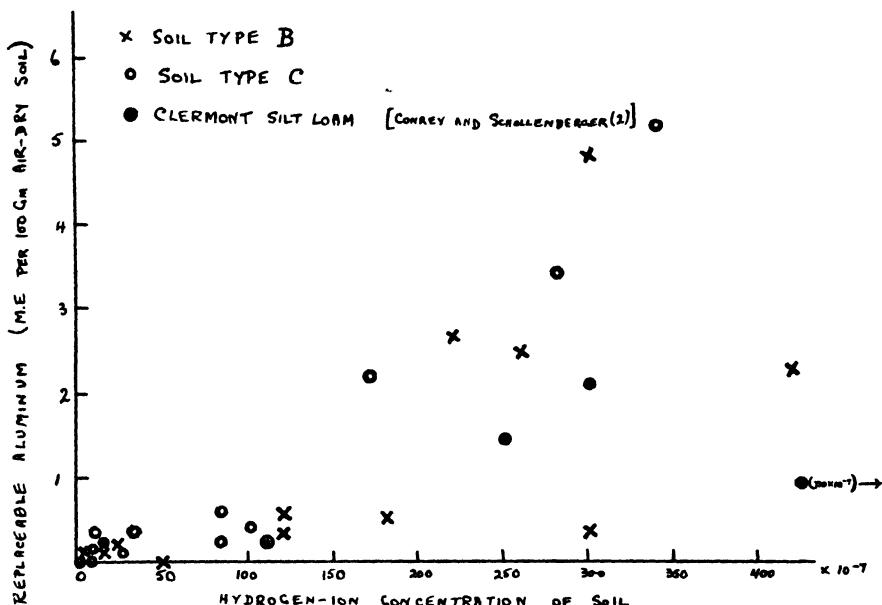
FIG. 1. RELATIONSHIP BETWEEN HYDROGEN-ION CONCENTRATION AND CONTENT OF REPLACEABLE ALUMINUM OF SOIL

tively low electro-affinity, would be replaced with difficulty by sodium ions. On the other hand, when exchange is entirely indirect through interaction with released hydrogen ions, replacement would not only be more rapid, but would tend to take place in the earlier stages of the leaching process, i.e., at the time when the filtrate possesses its maximum hydrogen-ion concentration.

It is worthy of note that Magistad (8) found the amount of aluminum present in the soil solution to increase rapidly at pH values below 4.8

Replaceable hydrogen

The saturation deficits of the soils examined are plotted against the amounts of replaceable aluminum in figure 2.

It will be seen from this figure that:

(a) The amount of replaceable aluminum bears a fairly close relationship to the soil content of replaceable hydrogen.

(b) The points tend to fall on two smooth curves, which may be distinct, and which correspond to the two soil types.

The data recorded by Conrey and Schollenberger (2), when similarly plotted, give rise to a third curve. It should be mentioned, however, that these workers used a normal solution of ammonium acetate as the leaching agent, and measured replaceable hydrogen by electrometric titration of the filtrate.
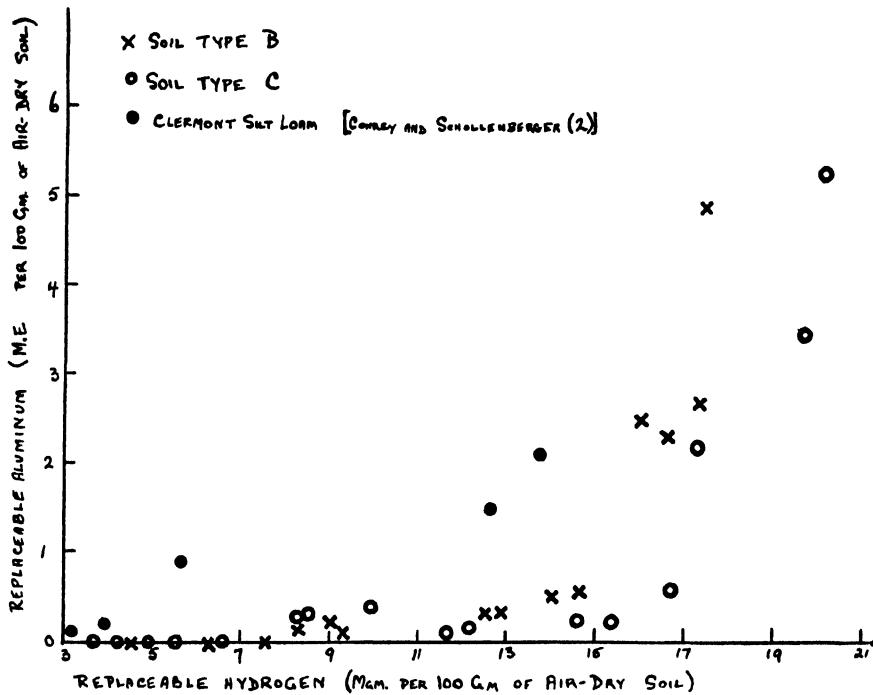
FIG. 2. RELATIONSHIP BETWEEN SOIL CONTENT OF REPLACEABLE HYDROGEN AND ALUMINUM

The existence of a different curve for each soil type is to be expected, for the data of Burgess (1) indicate that the amounts of aluminum dissolved by dilute acid from soils of approximately equal pH value, but belonging to different types, may vary greatly. Further, the degree of dehydration of the alumina gel in soils, which many consider to be the main source of the replaceable aluminum, may vary with soil type, and in consequence may exhibit different degrees of reactivity towards acid.

(c) Replaceable aluminum is not found in the filtrate until the saturation deficit attains a minimum value of 8.25 m.e.

The existence of such a minimum value suggests that a definite hydrogen-

ion concentration must be attained by the filtrate before aluminum becomes replaceable. It provides an explanation of the lack of consistency in the occurrence of aluminum in the filtrates obtained from soils with pH values greater than 5.1.

(d) The curves fall into two portions, one of which corresponds to the range of saturation deficit 8.25 to 15.5 m.e. and the other to the range 15.5 to 21 m.e. Between 8.25 and 15.5 m.e. each curve approximates to a straight line, the amount of aluminum replaced being small. When the deficit exceeds a value of 15.5 m.e. a very rapid and fairly regular increase is evident in the quantity of aluminum.

A saturation deficit as large as 15.5 m.e. is found only in the soils with pH values less than 5.1. It therefore appears probable that the amount of aluminum present in the normal salt extract is completely dependent on the magnitude of the saturation deficit, provided the pH value of the soil is greater than 5.1, and that, under such circumstances, the aluminum is replaced by indirect means alone. On the other hand, both direct and indirect replacement may occur at lower pH values.

It is unlikely that the curve for indirect replacement continues indefinitely as a straight line when the saturation deficit exceeds 15.5 m.e. It is difficult, therefore, to distinguish the aluminum which is indirectly replaceable from highly acid soils from that which may be directly exchangeable. As the total amount of aluminum replaced from such soils is more closely related to saturation deficit than to the pH value (fig. 1 and 2), the former is probably the more important controlling factor. This is borne out by the rapid fall in the content of replaceable aluminum which accompanies a decrease in saturation deficit (e.g., compare soil M 29 with M 23 and M 30). Presumably, therefore, the major portion of the aluminum passes indirectly into solution even in the soils of lowest pH value.

Similar considerations probably hold for the replacement of iron. In this case, however, a larger saturation deficit (10 to 12 m.e.) is needed before the base becomes indirectly exchangeable in measurable quantity. Also, as iron, when replaceable, passes into solution in the earlier stages of the leaching process (none was found in the filtrate samples taken at the end of the first liter stage of the extraction), and as no marked increase is evident in the contents of iron of the filtrates from the most acid soils, it is doubtful whether any of the soils of types B and C possess a pH value low enough for it to be present in the directly exchangeable form. Such a state of affairs would be in accordance with the results of Joffe and McLean (5), who found that iron is precipitated from solution at a higher hydrogen concentration than is aluminum.

It has been suggested that the aluminum passes into the salt extract as colloidal hydrous oxide from soils with pH values approaching neutrality. Under certain circumstances this assumption may be largely correct. Nevertheless, in view of the relationship which obtains between the initial appearance of aluminum in the filtrate and the magnitude of the saturation deficit

under stringent conditions of filtration, and as the filtrate attains its maximum hydrogen-ion concentration very rapidly (7), the importance of the assumption may be over-emphasized.

The extent to which the aluminum present in the filtrate could be responsible for the exchange acidity of the soils examined is a matter of some interest. The value of the ratio of replaced hydrogen to aluminum is of the order of 50 for soils with saturation deficits between 8.25 and 15.5 m.e. At greater deficits the ratio falls rapidly, reaching a minimum of 3.6 in soil M 23. The amount of aluminum in the filtrate is therefore not even approximately equivalent to the saturation deficit in the case of any of these soils, but the trend of the curves in figure 2 indicates that a state of equivalence is likely to exist in soils possessing slightly larger deficits than those recorded. These results are not in agreement with the theory that the replaced aluminum can account for the exchange acidity of soils of intermediate, rather than low, pH value.

Degree of unsaturation

As the necessary data were available, the relationship between the degree of unsaturation of the soil (14) and its content of replaceable aluminum was also investigated. The amounts of aluminum in the filtrates from the soils of both types were found to be smaller than 0.60 m.e. until the degree of unsaturation exceeds 60 per cent, when the aluminum increases substantially but irregularly in quantity. In all other respects the measure of correspondence between the two factors is small.

THIOCYANATE REACTION

Suggestions have been made, notably by Hissink (4), that the intensity of color developed when soils are treated with an alcoholic solution of potassium thiocyanate may be used as an indication of their degree of acidity. Saint (11), however, has shown that the relationship is not uniform in character. He ascribes the inconsistences to variation in the iron and organic matter contents, and to the texture, of the soils he examined.

In view of the practical value of the thiocyanate test, and its widespread use, advantage has been taken of the data accumulated to reinvestigate in more detail the relationship between the color intensity and certain soil factors.

The following procedure was used for determining thiocyanate color: 1-gm. samples of soil were shaken with 5 cc. of a 40 per cent alcoholic solution of potassium thiocyanate for 5 minutes. The suspensions were allowed to settle for 48 hours and the intensity of color developed was compared with that of standard solutions containing known amounts of iron. The figures obtained, which are based on arbitrary units, are set out in table 2.

Replaceable iron

In the soils examined the measure of correspondence between thiocyanate color and the amounts of iron replaced is small.

As traces only of iron were found in the majority of the filtrates an attempt was made colorimetrically to determine the amounts present, to enable them to be correlated with thiocyanate color. The attempt was unsuccessful, as the colors given by the filtrates in most cases were too faint to be differentiated accurately.

pH value

The soils with pH values greater than 6.0 gave no coloration with potassium thiocyanate. Those with pH values smaller than 6.0 gave a color, irrespective of the magnitude of their saturation deficits.
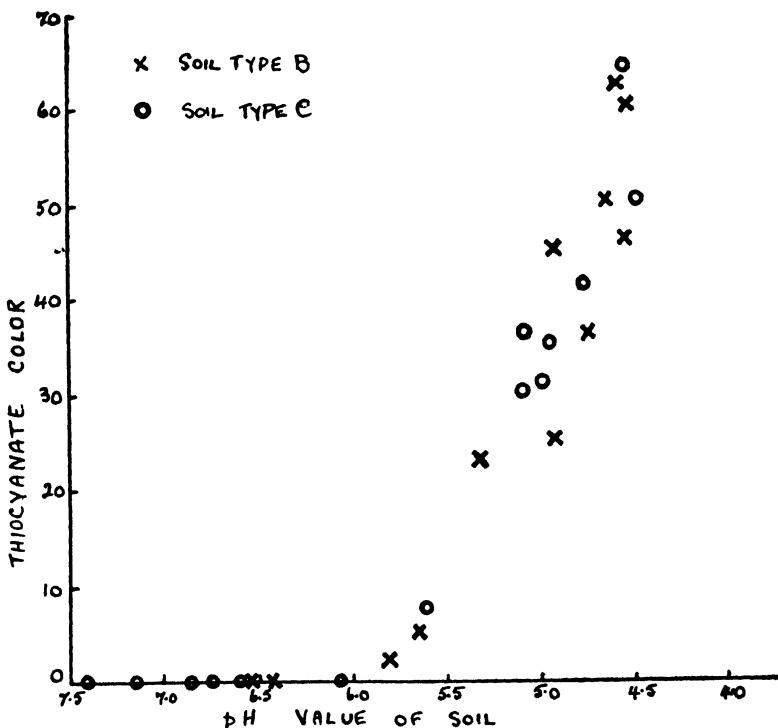
FIG. 3. RELATIONSHIP BETWEEN pH VALUE OF SOIL AND THIOCYANATE COLOR

The relationship between pH value and thiocyanate color is very close (fig. 3). The points fall on a straight line over the whole range of pH values recorded, for which colors are evident. The correlation between intensity of color and pH value is -0.938 for the 18 soils giving a positive reaction, P being smaller than 0.01.

When thiocyanate color is plotted against hydrogen-ion concentration as such, the tendency of the curve to bend over toward the hydrogen axis becomes marked in the region of an ion concentration of 1×10^{-5} (pH 5).

Replaceable hydrogen

The relationship between thiocyanate color and saturation deficit also appears linear (fig. 4), but it is not as well defined as that which holds for pH value.

The correlation coefficient between thiocyanate color and saturation deficit is + 0.761, with *P* less than 0.01.

Discussion

The lack of correspondence between the amounts of replaceable iron and thiocyanate color indicates that the conditions controlling replacement in the
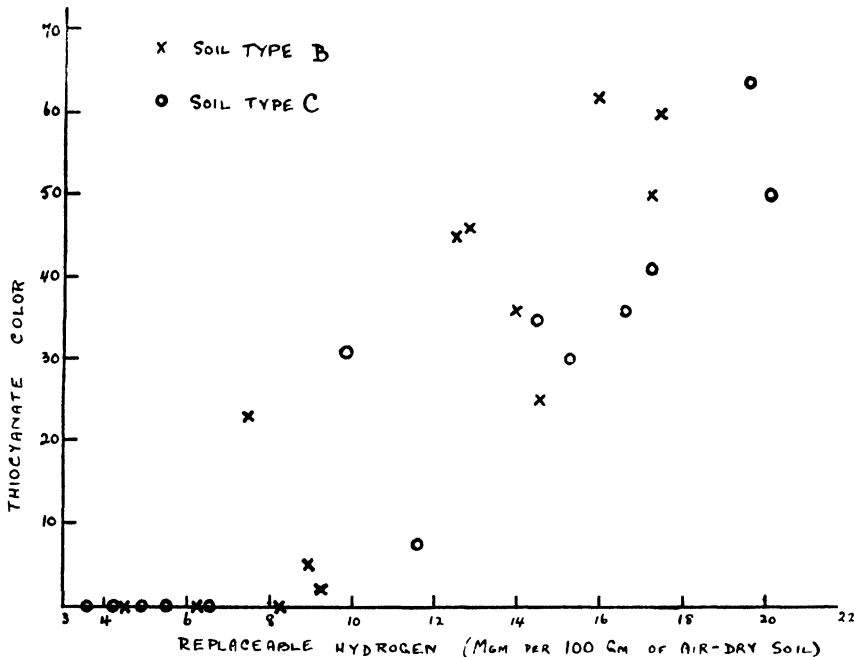
FIG. 4. RELATIONSHIP BFTWEEN SOIL CONTENT OF REPLACEABLE HYDROGEN AND THIOCYANATE COLOR

presence of sodium chloride and potassium thiocyanate differ materially. The high concentration of the thiocyanate solution, and the formation of a very slightly ionized ferric salt in this case, are undoubtedly contributing factors, for they tend to bring about maximum replacement under the conditions of the experiment, and to minimize the effect of disturbing influences due to the presence of other ions.

The relation between thiocyanate color and pH value at first sight appears anomalous. The high degree of correspondence is probably due to the fact that no attempt is made in the thiocyanate reaction to drive replacement to completion by continued leaching with fresh solution. In consequence, the

thiocyanate color is less dependent on the total exchange acidity of the soil, and more dependent on its immediately available acidity, than would be the case under the conditions of normal exchange procedure. The use of an alcoholic solution probably emphasises such a state of affairs by depressing the degree of ionization of the soil complex.

Statistical analysis of the data recorded lends confirmation to this view. The partial correlation coefficient between thiocyanate color and pH value, when allowance is made for saturation deficit, is -0.853 ($P = <0.01$). This value does not differ significantly from the total correlation coefficient. The partial correlation coefficient between color intensity and saturation deficit, pH value being constant, is $+ 0.215$ ($P = >0.1$). pH value is therefore undoubtedly more important than total saturation deficit (as measured by the method described) in determining thiocyanate color. The direct correlation coefficient between color intensity and saturation deficit over-estimates the degree of correspondence between these factors.

The multiple correlation coefficient between thiocyanate color, and pH value and saturation deficit, which measures, in a sense, the extent to which color intensity is related to their combined variation, is 0.941.

SUMMARY

An examination has been made of the influence which the hydrogen-ion concentration and the state of unsaturation of the soil exert upon its content of replaceable iron and aluminum. It has been found that:

Replaceable aluminum is invariably present only in soils with pH values less than 5 1. The relationship between the hydrogen-ion concentration of the soil and the quantity of replaceable aluminum is very irregular.

The amount of replaceable aluminum appears closely related to the magnitude of the saturation deficit. Aluminum is not found in the filtrate until the deficit attains a minimum value of 8 25 m.e. Until the deficit reaches 15 5 m.e., it increases slowly in quantity. Above 15.5 m.e. the increase is extremely rapid.

The amount of aluminum replaced at any given saturation deficit may vary with soil type.

Indications exist that replacement of aluminum is wholly indirect in soils with pH values greater than 5.1 (the maximum recorded pH value corresponding to a saturation deficit of 15.5 m.e.). At lower pH values direct replacement may also occur, but indirect replacement appears to predominate.

Between the deficits 8.25 and 15.5 m.e. the ratio of replaceable hydrogen to aluminum is of the order of 50. The rapid fall in the ratio at greater deficits suggests that the replaced aluminum may be equivalent in amount to the exchange acidity in highly unsaturated soils.

The measure of the relationship between the amount of replaceable aluminum and the degree of unsaturation of the soil is small.

Iron does not appear in measurable quantity in the filtrate until the saturation deficit attains a value of 10 to 12 m.e. None of the soils examined appear to possess a pH value low enough for iron to become directly exchangeable.

The relationship between thiocyanate color and soil acidity has been reinvestigated. It has been found that the intensity of color is more closely related to the pH value of the soil than to the saturation deficit.

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THE DETERMINATION OF LIME REQUIREMENT BY THE DIRECT ADDITION OF CALCIUM CARBONATE

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Since calcium carbonate is the material used most generally for the correction of soil acidity, it would seem that the best estimate of the lime requirement of a soil could be made in the laboratory by the use of $CaCO_3$.[2] Jensen (9), who used lime water and changed the excess to $CaCO_3$ by passing CO_2 through the soil suspension stated that a nearer approach to natural conditions would be made if the soils were mixed directly with $CaCO_3$ or a solution of $Ca(HCO_3)_2$. Biroux and Pien (2) added the calculated amount of lime and three times the calculated amount to samples of soil in the laboratory and after making them up to optimum moisture let them stand. The results showed a slow reacidification, some soils becoming more acid than at the start, after standing 8 weeks. Mattson (12), using an electrodyalized Sharky colloidal material (11) placed his 1-gm. samples in conical bags of thin parchment paper with enough water completely to saturate and cover the soil. The bags were then placed in a soft paste of pure $CaCO_3$ and at intervals of 1, 3, 7, 8, and 14 days a bag was removed and its contents tested for adsorbed calcium. Seven days were required for equilibrium when the soil material was completely unsaturated.

Crowther and Martin (6) added 2 gm. of $CaCO_3$ to 0, 10, 20, 30, and 40 gm. of soil in uniform 275-cc. bottles, filled the bottles with water and a drop or two of toluene and after shaking 16 hours let them sit for a day or two till reasonably clear. Then they determined the total carbonic acid both as free and as bicarbonate and found it to be greater than that estimated by the Hutchinson-MacLennan method.

Demalon (7) obtained pH 7.1 by adding powdered $CaCO_3$ to the soil suspension in a Knight shaking electrode where the CO_2 was eliminated by the bubbling H.

EXPERIMENTAL

Several 60-gm. samples of the soil, which was air-dry and had been passed through a 1-mm. sieve, were weighed out. To these were added varying

amounts of c.p. $CaCO_3$, from 0.06 gm., representing 1 ton of $CaCO_3$ to the acre of soil weighing 2,000,000 pounds, to 0.48 gm. representing 8 tons of $CaCO_3$ to the acre.   In order to maintain the same water relation as is used in the electrometric determinations of pH, 60 cc. of water was added to each sample.   In the first experiment mixing was accomplished in tightly stoppered 250-cc. widemouth bottles using an end-over-end shaker.   The course of the reaction was followed by determining the pH of the suspension by the quinhydrone electrode.   Equilibrium was reached in 20 to 24 hours.   The results for the method samples are shown in curve I, figure 1.   As the failure to obtain neutrality was
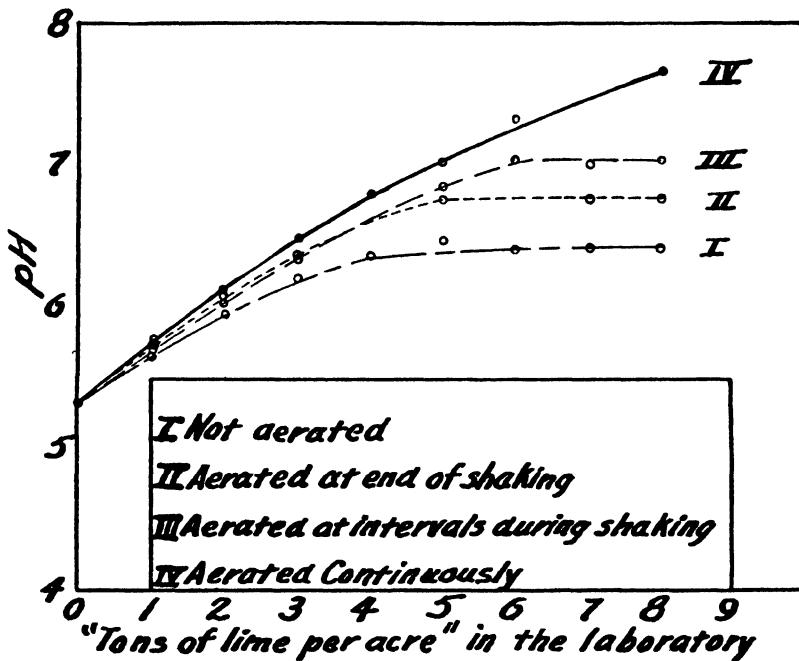
FIG. 1. THE EFFECT OF AERATION ON THE TITRATION CURVES WHEN $CaCO_3$ IS USED

probably due to the accumulation of CO_2, an upright shaker was used, the bottles were left unstoppered, and the suspension was aerated.

Curve II, figure 1, was obtained by this method with aeration only at the end of the shaking period; curve III with aeration at intervals during the shaking.

Finally, in order to remove CO_2 as fast as it was formed, the equipment shown in plate 1 was used.   The air drawn through the series of funnels by a water aspirator was passed through water before entering the first funnel in order to avoid drying out the sample in the first funnel.   The stopcocks were adjusted so that sufficient air passed through to keep the soil in motion and to prevent the soil suspension from entering the stems of the funnels.   Equilibrium was reached in about 20 hours.   The results obtained are readily reproducible. Curve IV, figure 1, illustrates the results with the method sample.

This method was used on the soils listed in table 1. The results are presented in table 2 and the titration curve for soil IV, highly buffered, and soil II, poorly buffered, are shown in figure 2. The lime requirement was calculated

Soil II Poorly Buffered
Soil IV Highly Buffered

"Tons of lime per acre" in the laboratory
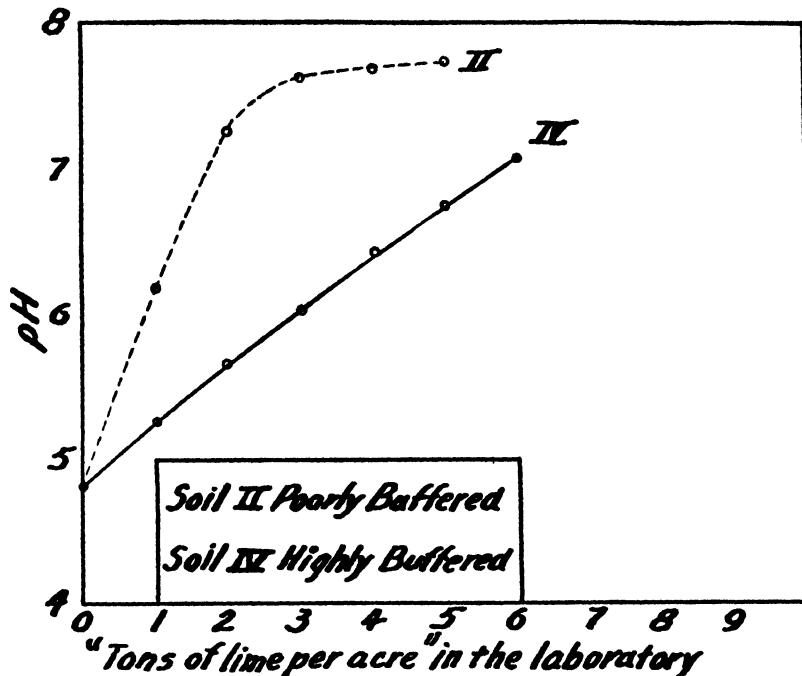
FIG 2 TITRATION CURVES OF TWO SOILS THAT DIFFER IN ORGANIC MATTER

TABLE 1

Description of the soils used in this experiment

NAME OF FARM	NUMBER OF SOIL	TYPE OF SOIL			pH
		Sand	Silt	Clay	
		per cent	per cent	per cent	
Adams	I	20 0	25 4	54 6	5 186
Carter	II	68 3	17 7	14 0	4 837
Currier	III	74 7	9 7	15 6	5 894
Jackson	IV	47 2	26 0	26 8	4 805
Livingstone	V	47 4	33 7	18 9	5 634
Redden	VI	71 0	10 4	18 6	5 650
Whenal	VII	57 9	20 4	21 7	4 873

by interpolation as the number of tons of $CaCO_3$ required to bring 2,000,000 pounds of the soil to pH 7.0.

The lime requirement of these soils was determined also by the following methods: pH as determined by the quinhydrone electrode in a 1–1 water sus-

TABLE 2

The results obtained in the laboratory from the use of CaCO$_2$

NUMBER OF SOIL	TONS OF LIME IN LABORATORY						
	0	1	2	3	4	5	6
	pH	pH	pH	pH	pH	pH	pH
I	5.186	5 648	6 071	6 414	6 867	7 227	
II	4 837	6.196	7 248	7 632	7.706	7.741	
III	5 894	6 445	6 950	7 356	7 690	7 871	
IV	4.805	5 250	5 670	6 038	6 408	6 721	7.075
V	5 634	6.162	6 712	7 161	7 558	7 696	
VI	5 650	6 244	6 754	7 221	7.631	7.724	
VII	4 873	5 487	5 931	6 319	6 692	7.044	

TABLE 3

A comparison of the results obtained by different lime requirement methods

SOIL	TONS OF CaCO$_2$ AS DETERMINED BY THESE TESTS					
	CaCO$_2$	Truog	Jones	pH*	Hydrolytic acidity	Exchange acidity
I	4 4	4 0	3 4	4 3	3 9	0 7
II	1.8	2 0	1 6	3 8	1 9	0 3
III	2 1	1 5	2 1	1 25	2 2	0 05
IV	5 8	4 0	3 9	4 5	4 5	0 3
V	2 6	1 0	2 3	2 5	2 6	0 0
VI	2 5	1 0	2 1	1 75	2 8	0 1
VII	4 9	3 0	3 4	4 0	4 2	0 5

* Lime requirement as determined by the pII was based on the type of soil as determined by the Bouyoucos hydrometer method (1) and a table prepared by Morgan (13), using alfalfa as the test crop.

TABLE 4

The results obtained in the field from the application of ground limestone

NUMBER OF SOIL	TONS OF LIME IN FIELD							
	1		2		3		4	
	pH	L F *	pH	L F	pH	L F.	pH	L F.
I			5 70	1 6				
II	5 63	1.30	6 08	2.22	6 39	2 55		
III	6.52	0.87						
IV			5 54	1 45			5 81	1.66
V	5 91	1.90	6 18	1 90				
VI			6 25	2 00				
VII			5 97	0.98			6 42	1 23

* L.F. = liming factor.

pension, Truog (18), Jones (8), hydrolytic acidity (10), and exchange acidity (10). The last two were calculated on the basis of an acre of soil weighing 2,000,000 pounds. The results obtained are given in table 3.

The results of the addition of ground limestone in the field to the soils studied are presented in table 4. These figures represent the maximum change which occurs in most of the cases 2 years after application and which agrees with Rost and Fieger (15).

<center>DISCUSSION</center>

The results in table 3 show a marked difference in the amount of lime to be added as determined by the various methods. Runk (16) compared the Comber, Truog, and sodium acetate tests and obtained varying results. Since these tests measure different forms of acidity a difference would be expected. The $CaCO_3$ method is used as a basis of comparison.

The Truog method gives lower results, with the exception of soil II. This soil was low in organic matter. There was more than a ton's difference on some of the farms, and as these results were consistently low and since there was a personal element involved in reading the color, the Truog was considered unsatisfactory.

The Jones method also gave low results even though the factor 1.8 was used. However, this may be due to the need of a larger factor for New Hampshire soils. The results are better than with the Truog method, and, with a factor that varied with the type of soil, better results would be obtained.

The lime requirement as determined by the pH was based on the type of soil as determined by the Bouyoucos hydrometer method (1) and a table prepared by Morgan (13), using alfalfa as the test crop. The results are for the most part better than the Jones method with the exception of soil II, which is 2 tons too high.

The hydrolytic acidity results agreed best with the $CaCO_3$ figure. As in the Jones test, calcium acetate was used, but the longer and more thorough mixing with the soil, together with the different method of calculation, accounts for the improved results.

From the exchange acidity results it is evident that New Hampshire soils contain very little exchange acidity and in the majority of cases it can be disregarded as a factor to be considered when a field is to be limed. Biroux and Pien (2) found the exchange acidity as determined by the Daikuhara method to be in the great majority of cases 10 to 20 times lower than the electrometric and Hutchinson method.

The $CaCO_3$ method was carried out with field experiments as standards [cf. Jensen (9) and Crowther (4)]. Jensen (9) found that he had to multiply his laboratory results by three in order to obtain the same effect in the field, which gave him a "liming factor" of 3. Pierre and Worley (14), who limed pots in the greenhouse with amounts of $CaCO_3$ based on the soils' exchange hydrogen, did not obtain pH 7 and decided that either the $CaCO_3$ reacted with other materials

or not all acid was determined. They also stated that the non-exchangeable soil complex was believed to be responsible for the presence of the "liming factor" of 1.5 which they obtained. Crowther (5) believed that the liming factor was due to considerable movement of lime into the subsoil.

Table 4 shows the field results and the liming factors. Since the soils were not limed to pH 7.0 the factors were figured for the actual pH obtained. Conner, Walker, and Plice (3) had to use 12 tons of limestone to obtain pH 7.1 on a Clermont silt loam, and with a Newton sandy loam 16 tons gave them only pH 6.5. Our field results tend in the same direction and since good yields can be obtained without completely neutralizing the soil [cf. Slipher (17), Runk (16), and Conner, Walker, and Plice (3)], one can, by using the liming factor and laboratory curve, lime to a definite pH under 7.0. As shown in table 4 the liming factor varies with the different soils and with different amounts of lime in the same soil.

Soil II, which had the steepest laboratory curve (fig. 2), gave the greatest "liming factor." However, there was a definite increase in the weight of silage corn grown on this field with each added ton of lime. The soil being sandy and poorly buffered, one would expect more change in pH in the field. The subsoil has not been checked and the lime may have been carried there or passed through as nitrate, sulfate, and chloride [cf. Conner, Walker, and Plice (3)].

For the most part the factor varies between 1 and 2. Soils III and VI, which are both high in sand but with VI having more organic matter, have factors of 0.87 and 2.00 respectively. Soils IV and V, which are fairly high in organic matter also have a high factor whereas soil VII, which is low in organic matter, has a factor near 1.0. Soil I is very high in clay, which acts as a buffer, and has a factor similar to a soil that is high in organic matter.

SUMMARY

A method of obtaining the lime requirement by the direct use of $CaCO_3$ has been described.

From interpolation on the laboratory curve and by the use of a factor depending on the amount of organic matter, it is possible to calculate how much lime to apply in the field in order to obtain a definite pH. This is a decided advantage as it is not necessary to lime to pH 7.0., and various crops require different pH values.

The $CaCO_3$ method agrees favorably with the hydrolytic acidity method for determining lime requirement.

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PLATE 1

THE EQUIPMENT USED TO MIX THE CaCO₄ WITH THE SOIL SUSPENSION
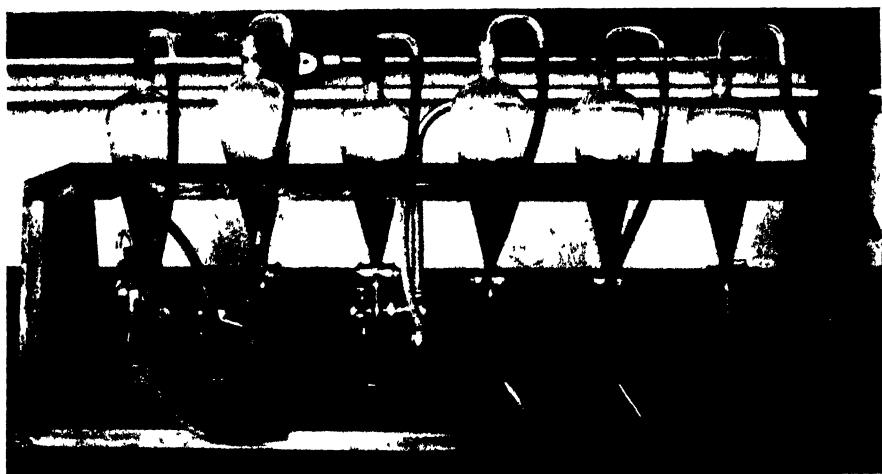
EFFECTS OF SORGHUM PLANTS ON BIOLOGICAL ACTIVITIES IN THE SOIL

ARTHUR D. McKINLEY

Iowa State College

Fortunately, the conditions necessary for the normal development of the majority of higher plants and the beneficial forms of microorganisms in the soil are approximately the same. Experiments have definitely proved that neither group of organisms can function normally for any extended period of time without the other. All life is dynamic and subject to the influences of many other things, of which one of the more important is other types of life. That growing plants and microorganisms in the soil may affect each other, either favorably or unfavorably, is now an accepted fact. Some of the relationships between the two groups of organisms have been demonstrated satisfactorily, whereas others are, as yet, but incompletely worked out and not well understood.

Starkey (8) recently discussed the relationships between higher plants and microorganisms in the soil and gave a comprehensive review of the literature on the subject. The review of literature shows that the evolution of carbon dioxide is greater from soils that support plant growth and that the amounts of gas produced depend on the kinds of plants and the character of their growth. Maximum effects have been found at advanced stages of vegetative development and fruiting Correlated with carbon dioxide production are numbers of microorganisms which are increased on the roots and in the vicinity of the roots of many crops, the most striking increases being in the *B. radiobacter* group, although the general bacterial population, actinomycetes, and filamentous fungi all show increases. Plant roots have been found to excrete soluble organic substances and the increased numbers of oganisms on and near the roots appear to be due, largely, to root excretions and not to decaying root cells. The presence of legumes apparently stimulates bacterial activities in the soil to a greater extent than the presence of non-legumes, this being especially true of nitrification.

There are few data concerning the effects of sorghum plants on the biological and chemical activities in the soil. The following work with kafir, corn, wheat, and barley was undertaken to determine whether sorghums affect soil activities more or less than corn or other crops.

Studies were carried on in both cropped and fallowed soils and determinations were made of carbon dioxide evolution, the nitrate content of the soils, the hydrogen-ion concentration of the soil solutions, the total numbers of filamentous fungi and other microorganisms (bacteria and actinomycetes), the water-soluble organic matter and water-soluble phosphorus content of the soil solution, and the nitrifying, nitrate assimilating, and nitrogen fixing powers of the soils.

The plants were grown in 4-gallon earthenware pots in the greenhouse. Clarion loam was used, each pot containing approximately 30 pounds. Four pots were planted to each of the four crops and four were left unplanted. Half of the pots were used for biological and chemical studies and the other half for carbon dioxide determinations. When the plants had developed sufficiently for the elimination of the weaker ones they were thinned, three corn or kafir plants and 21 wheat or barley plants being left in the respective pots.

The soils were kept near the optimum moisture content except for a few days prior to sampling, when water was withheld to allow the soils to become dry enough to permit sampling with a tube. After samples were taken, soil from the surface of the pot was worked and pressed into the holes and the moisture content was brought to optimum again. Soil, of the original supply, was added occasionally after sampling to keep the pots full and prevent exposure of the roots.

As the period of daylight at the time of planting was short, the pots were grouped in circles under reflectors having 500-watt Mazda bulbs. During December and the early part of January the lights were turned on in the late afternoon and turned off about 11 o'clock at night. The barley plants grew normally and corn responded well to the light, but wheat had a tendency to grow rapidly without the production of tillers. Kafir showed a marked indifference to the added light, although, judging from work with plants during the preceding winter, the increased amount of light kept the plants growing and prevented early maturity.

The barley plants were attacked by the powdery mildew, *Erysiphe graminis*, and were treated with powdered sulfur. All of the pots were dusted with sulfur so that comparative results could be obtained.

Soil samples for biological and chemical determinations were taken from the one group of pots at intervals of two weeks throughout the periods of growth and at maturity of the corn, wheat, and barley. The last soil samples were taken from the kafir pots when the plants were in full bloom. Soil samples were obtained by means of a small brass tube with an inside diameter of 12 mm. From 18 to 20 samples were taken from each pot. No attempt was made to obtain the samples under aseptic conditions, as the errors due to contamination were regarded as negligible.

The following methods were used in making biological observations and chemical determinations on the soils:

In the study of carbon dioxide evolution, samples of the soil atmosphere were obtained by means of glass aspirator bulbs surrounded with glass wool and buried in the soil about 2 inches above the centers of the bottoms of the pots. The bulbs were connected by means of glass and rubber tubing to a simplified Haldane apparatus which employed 50 per cent potassium hydroxide solution as the carbon dioxide absorbent.

The phenoldisulfonic acid method, as modified by Harper (3), was used in determining nitrate nitrogen.

The quinhydrone electrode was used in determining pH values.

Soil dilutions and plate counts of microorganisms were made according to the methods recommended by Waksman (10, 11). Sterile physiological salt solution (8.5 gm. of sodium chloride in a liter of distilled water) was used instead of tap water in making the dilutions.

Sodium albuminate agar (2, medium 5) was used in making plate counts of bacteria and actinomycetes.

Determinations of numbers of filamentous fungi were made with peptone-glucose acid agar (2, medium 18). The medium was adjusted to pH 3.8–4.0. Calculated amounts of N. H_2SO_4 were added before sterilization, no trouble being experienced in obtaining a firm medium of the desired pH value.

The method described by Bondorff and Christensen (1) was employed in making the determinations of water-soluble organic matter. Fifty grams of soil and 500 cc. of distilled water were used to obtain the filtrate.

Water-soluble phosphorus determinations were made according to the Deniges method, as modified by Parker and Fudge (6). The solutions for the tests were obtained by placing the equivalent of 25 gm. of air-dried soil and 50 cc. of distilled water inside a collodian bag. The bag was then immersed in 200 cc. of distilled water and the soil was dialyzed from 30 to 42 hours.

CARBON DIOXIDE EVOLUTION

The soils used in making the carbon dioxide determinations had stood in the pots throughout the previous summer and had become quite dry. They were planted on December 4 and the first carbon dioxide determinations were made on December 5. The dates of sampling and the results obtained are shown in table 1. The addition of water to the more or less dry soil at the beginning of the experiment brought about increased microbiological activities and a marked increase in carbon dioxide in the soil air. The amounts of carbon dioxide increased gradually until the maximum was reached, in most cases, on the third day. From then on there was a gradual decrease until January 30, when the lowest levels were reached. During the period from December 4 to December 30 the percentage of carbon dioxide in the soil atmosphere varied widely and there were, apparently, very minor effects from the plant roots. From December 30 to February 2 the amounts of carbon dioxide found in the soils varied, but the differences were not marked. There was evidence that the growing plants, with the exception of kafir, were causing increases in the amount of carbon dioxide. Beginning with February 2, when wheat and barley were heading and the corn tasseling, it was evident that all plants were bringing about increases in the amounts of carbon dioxide. The largest amounts of carbon dioxide were found under corn. Wheat had about the same effects as barley. The results obtained with kafir fluctuated widely until April 6, when marked increases in the amounts of carbon dioxide produced became evident.

TABLE 1

Carbon dioxide in the soil atmosphere under corn, kafir, wheat, barley, and fallow

PERCENTAGE BY VOLUME OF CARBON DIOXIDE AT DIFFERENT DATES OF SAMPLING

CROP	Decem ber 5	Decem ber 6	Decem ber 7	Decem ber 8	Decem ber 9	Decem ber 10	Decem ber 11	Decem ber 12	Decem ber 14	Decem ber 16	Decem ber 18	Decem ber 20	Decem ber 22	Decem ber 24	Decem ber 30	January 5	January 12
Corn	3 75	5 60	6 00	7 20	5 80	6 00	3 80	2 60	1 40	0 95	0 25	0 35	0 35	0 25	0 10	0 20	0 30
Corn	4 40	5 80	5 60	4 90	3 20	2 80	1 40	0 90	0 60	0 40	0 20	0 15	0 20	0 20	0 05	0 20	0 20
Kafir	3 50	4 30	3 90	3 00	1 70	1 80	0 80	0 60	0 40	0 40	0 15	0 15	0 17	0 22	0 02	0 17	0 17
Kafir	4 20	5 80	5 30	4 40	2 40	2 40	1 00	0 70	0 45	0 40	0 20	0 20	0 15	0 25	0 10	0 20	0 17
Wheat	3 10	4 00	4 20	3 80	3 40	3 85	2 60	2 00	1 35	0 60	0 30	0 20	0 20	0 22	0 10	0 17	0 20
Wheat	3 60	4 80	5 00	4 70	4 20	4 15	2 40	1 70	0 95	0 60	0 20	0 20	0 25	0 25	0 10	0 15	0 22
Barley	3 80	5 30	5 70	5 20	5 00	5 25	3 00	1 80	0 70	0 60	0 20	0 20	0 20	0 20	0 10	0 17	0 30
Barley	3 60	4 80	4 60	4 00	3 00	2 75	1 45	1 00	0 60	0 25	0 20	0 15	0 25	0 22	0 05	0 25	0 25
Fallow	4 20	4 60	3 60	2 40	1 40	1 05	0 50	0 25	0 20	0 15	0 10	0 15	0 20	0 17	0 05	0 25	0 20
Fallow	3 45	4 90	4 70	5 40	4 10	5 10	3 70	2 75	1 70	1 05	0 35	0 40	0 40	0 40	0 05	0 17	0 17

PERCENTAGE BY VOLUME OF CARBON DIOXIDE AT DIFFERENT DATES OF SAMPLING

CROP	January 19	January 26	Febru ary 2	Febru ary 9	Febru ary 16	Febru ary 23	March 2	March 4	March 9	March 16	March 23	March 30	April 6	April 13	April 24	May 3	May 8
Corn	0 22	0 30	1 80	0 60	1 30	1 62	0 42	1 20	2 40	1 85	1 45	0 90	2 30	1 45	1 10		
Corn	0 20	0 20	0 90	0 90	1 35	1 00	0 25	1 20	3 20	1 75	1 40	0 77	1 80	1 60	1 30		
Kafir	0 15	0 10	0 35	0 20	0 80	0 55	0 20	0 42	0 55	0 80	0 75	0 70	1 60	2 60	0 85	0 95	0 90
Kafir	0 15	0 15	0 37	0 20	0 75	0 45	0 22	0 60	1 00	0 75	0 85	0 80	1 40	1 90	1 05	1 35	1 25
Wheat	0 20	0 22	0 40	0 57	1 60	0 37	0 10	0 40	0 42	0 50	0 60						
Wheat	0 22	0 20	0 80	0 55	1 00	0 42	0 15	0 60	0 82	0 50	0 47						
Barley	0 32	0 35	0 62	0 55	1 40	0 52	0 22	0 60	0 80	0 55	0 55						
Barley	0 22	0 22	0 60	0 55	1 30	0 50	0 22	0 50	0 45	0 40	0 45						
Fallow	0 10	0 10	0 17	0 17	0 20	0 12	0 20	0 20	0 05	0 20	0 20	0 15	0 20	0 20	0 20	0 20	0 20
Fallow	0 15	0 10	0 20	0 20	0 25	0 25	0 05	0 20	0 10	0 22	0 20	0 15	0 20	0 20	0 15	0 15	0 20

It is of interest to note the marked differences in the results obtained at times from the duplicate pots. The results obtained on March 2 are also of interest, as a very cloudy and cold period occurred at that time. The cold, cloudy weather evidently caused a depression in both plant and microbiological activities which was reflected in the carbon dioxide content of the soil atmosphere.

<div align="center">SOIL NITRATES</div>

Determinations for nitrates were made on 50-gm. equivalents of air-dried soil. The results are shown in table 2. The amounts of nitrates in the soils at the time of planting varied considerably. No marked changes in the nitrate content took place in the corn soils during the first eight weeks, but at the end of the tenth week or about two weeks before the plants began to tassel a de-

<div align="center">TABLE 2</div>
<div align="center">*Nitrate nitrogen content of soil under corn, kafir, wheat, barley, and fallow*</div>

CROP	NITRATE NITROGEN P P M											
	November 22	December 6	December 20	January 3	January 17	January 31	February 14	February 28	March 14	March 28	April 11	April 25
Corn	49 7	50 0	47 1	43 5	50 0	38 2	31 7	37 2	40 8	41 6	40 6	
Corn	41 8	41 3	41 3	41 3	38 4	25 6	10 8	11 2	5 9	11 1	18 5	
Kafir	38 8	43 4	45 4	41 9	47 6	47 6	42 2	49 6	51 0	43 5	38 5	25 6
Kafir	49 3	50 0	54 2	54 3	61 0	59 5	60 3	55 1	62 3	55 5	44 6	44 6
Wheat	34 1	35 9	37 0	28 6	38 2	34 9	32 5	38 1	51 0			
Wheat	44 9	49 5	47 1	41 9	55 5	47 1	40 0	52 9	62 3			
Barley	34 9	27 7	25 0	24 3	23 5	17 4	11 9	17 0	24 0			
Barley	38 8	34 8	33 5	38 4	32 5	22 0	22 6	31 8	52 0			
Fallow	44 2	48 0	44 6	50 0	60 9	58 1	61 5	66 6	77 7	71 4	62 5	71 4
Fallow	39 0	38 4	37 6	45 4	50 0	50 5	48 4	61 5	63 5	71 4	55 5	60 9

cided decrease was evident. A further decrease was noted at the end of the twelfth week. From then on to harvest the soils in the duplicate pots behaved differently. The nitrate content of the kafir soils behaved in a very erratic manner, increasing to the end of the sixteenth week and then decreasing during the next six weeks. The smallest amounts were obtained at the last sampling when the plants were in full bloom. The nitrate content of the wheat pots showed considerable fluctuation from week to week, but there was no evidence of the plants having utilized large amounts at any particular period. The smallest amounts of nitrates were found about four weeks before heading and at blossoming time. The nitrate content of the barley soils was lowest at the end of the twelfth week (blossoming time) after which an increase occurred. The fallow soils gained and lost in nitrate content in an irregular way until the end of the experiment, when there was about twice as much nitrate nitrogen

as at the beginning.   It is evident that the plants made their greatest draft on soil nitrates prior to and at the time of fruit formation.   Wheat did not draw so heavily on soil nitrates as did the other crops.   Crop growth was evidently not heavy enough to utilize the nitrates much faster than they became available.

HYDROGEN-ION CONCENTRATION

Hydrogen-ion determinations were made along with the other determinations and although differences were found from time to time the data did not indicate that the plants caused definite changes in pH values at any particular stage of their growth.   The data in table 3 show that growing plants tend to cause a decrease in hydrogen-ion concentration compared to that of unplanted soils, corn and kafir having much greater effects than wheat or barley.

TABLE 3

Hydrogen ion concentration of soil solutions under corn, kafir, wheat, barley, and fallow

CROP	pH VALUES AT DIFFERENT DATES OF SAMPLING*			
	December 4	March 28	April 25	May 9
Corn	7 19		7 41	
Corn	7 21		7 70	
Kafir	7 18			7 51
Kafir	7 17			7 51
Wheat	7 17	7 12		
Wheat	7 19	7 32		
Barley	7 20	7 29		
Barley	7 21	7 29		
Fallow	7 19			6 92
Fallow	7 19			6 99

* Soil samples from CO_2 pots.

NUMBERS OF BACTERIA AND ACTINOMYCETES

Bacteria and actinomycetes appeared to grow equally well on the medium used.   As so many of the colonies were small, necessitating the use of the microscope to differentiate between bacteria and actinomycetes, it seemed preferable to count the two orders together rather than make a higher dilution and increase the chances for error.   The numbers found at different dates of sampling are recorded in table 4

The numbers of bacteria and actinomycetes fluctuated widely from time to time and in duplicate pots.   The organisms began to increase in numbers during the latter part of January and, with few exceptions, the numbers remained relatively high until the end of the experiment.   That the increased numbers were not entirely due to the influence of the growing plants is shown by the fact that marked increases occurred in the fallow soils.   By averaging the numbers of bacteria and actinomycetes for 10 counts, or a period of 18 weeks,

the corn soils were found to have an average of 10,761,000 organisms per gram of soil; kafir soils averaged 9,258,000; wheat soils 9,720,000; barley soils 9,101,-000; and fallow soils 8,449,000. The corn soils averaged 12,863,000 bacteria and actinomycetes per gram of soil against 9,307,000 per gram of fallow soil during a period of 22 weeks. The kafir soils were found to average 11,466,000 bacteria and actinomycetes per gram of soil, whereas fallow soils averaged 9,716,000 for a period of 24 weeks. There was a remarkably close agreement between the average results of the duplicate treatments.

The foregoing figures show that larger numbers of bacteria and actinomycetes occurred in cropped than in uncropped soils, the largest numbers being found in corn soils. Root cutting by the soil sampling tube was, undoubtedly,

TABLE 4

Numbers of microörganisms (bacteria plus Actinomycetes) in the soil under corn, kafir, wheat, barley, and fallow

CROP	NUMBERS OF MICROÖRGANISMS PER GRAM OF SOIL AT DIFFERENT DATES OF SAMPLING*												
	November 22	December 6	December 20	January 3	January 17	January 31	February 14	February 28	March 14	March 28	April 11	April 25	May 9
Corn	114	576	636	808	736	806	520	2,428	2,100	1,962	2,837	1,924†	
Corn	86	1,034	596	696	748	1,890	836	1,604	1,546	1,802	2,984	1,604†	
Kafir	80	822	584	482	712	840	882	1,855	1,120	1,737	2,062	1,674	1,390†
Kafir	82	1,132	632	638	566	736	1,000	1,950	1,306	1,360	3,384	1,460	1,300†
Wheat	70	1,226	906	516	714	822	1,272	2,040	1,478	1,065†			
Wheat	74	876	590	728	702	760	1,057	2,164	1,496	885†			
Barley	64	854	422	506	486	1,206	1,080	1,916	1,470	1,130†			
Barley	94	608	472	638	552	862	1,440	1,682	1,227	1,494†			
Fallow	86	1,128	478	442	460	778	1,040	1,624	1,238	1,517	1,404	1,112	1,352†
Fallow	54	882	476	482	382	626	1,333	1,166	1,262	1,444	1,642	1,282	1,574

* Ten thousands omitted
† Soil samples from CO_2 pots.

responsible for part of the increases in cropped soils, but an examination of the root systems of the plants showed that root pruning had added relatively small amounts of organic matter to the food supply of the organisms at any one sampling. The root systems of the kafir plants were as well developed as, or better developed than, those of the corn plants, but fewer organisms were found under the kafir plants.

NUMBERS OF FILAMENTOUS FUNGI

The numbers of filamentous fungi obtained by plating the soils from under the various crops are recorded in table 5. The numbers fluctuated so widely from time to time and in duplicate pots that little information can be obtained

from an examination of the table. By averaging the numbers for all periods and for duplicate pots the corn soils were found to have an average number of 29,000 per gram of soil, while the fallow soil for a similar period averaged 26,530. The kafir soil averaged 22,060 filamentous fungi per gram of soil against 27,110 per gram for the fallow soil during a period of 24 weeks. For 10 samplings, or a period of 18 weeks, the average number per gram of soil was 26,930 under corn, 20,490 under kafir, 22,260 under barley, and 23,760 in fallow soil. Apparently, the numbers of filamentous fungi increased under corn and decreased under kafir, wheat, and barley. What the effects of root pruning, in the process of soil sampling, may have been is not clear, but the effects were not marked or larger numbers of filamentous fungi would have been found in

TABLE 5

Numbers of filamentous fungi in the soil under corn, kafir, wheat, barley, and fallow

CROP	NUMBERS OF FILAMENTOUS FUNGI PER GRAM OF SOIL AT DIFFERENT DATES OF SAMPLING*												
	November 22	December 6	December 20	January 3	January 17	January 31	February 14	February 28	March 14	March 28	April 11	April 25	May 9
Corn	68	282	334	252	332	270	432	340	252	286	620	334†	
Corn	76	474	162	224	296	152	342	236	244	332	482	282†	
Kafir	70	212	188	188	208	186	332	220	160	296	312	376	272†
Kafir	60	168	174	190	184	202	228	254	174	204	456	206	216†
Wheat	28	252	216	116	186	176	312	236	288	284†			
Wheat	98	242	244	148	194	186	318	332	238	166†			
Barley	54	226	158	192	150	348	340	318	322	186†			
Barley	68	162	162	172	202	234	332	306	260	260†			
Fallow	80	198	228	164	164	242	450	284	368	190	504	300	310†
Fallow	56	290	162	158	210	222	426	258	376	226	654	246	284†

* Hundreds omitted
† Soil samples from CO_2 pots.

the kafir, wheat, and barley soils. The average numbers indicate that corn produced organic materials in considerable quantities and of such a quality as to cause a significant increase in numbers of filamentous fungi. No reason is offered for the decreased numbers of filamentous fungi under other crops, except that the higher plants and other organisms may have competed for certain mineral elements to the disadvantage of the filamentous fungi. Such a theory, however, appears to be untenable in the case of nitrates.

WATER-SOLUBLE ORGANIC MATTER

The amounts of water-soluble organic matter in the soil solutions were determined every four weeks throughout the experiment. No differences were found among the soils at any one sampling, although the results varied

a little from one sampling to another. The results obtained showed no evidence that growing plants affect the amount of water-soluble organic matter in the soil. This is to be expected, for if plants do excrete readily decomposable organic substances, they would be quickly oxidized and there would be no accumulation of such materials.

WATER-SOLUBLE PHOSPHORUS

Determinations were made every four weeks for inorganic and total water-soluble phosphorus. The soils were rich in available calcium and no trace of inorganic phosphorus could be found in any of the samples. Small differences were found in the amounts of organic phosphorus, but they were not consistent and were within the limits of experimental error. As would be expected, none of the cropped soils showed evidence of the plants having materially increased or decreased the amounts of organic phosphorus in the soil.

NITRIFICATION, NITRATE NITROGEN ASSIMILATION, AND NITROGEN FIXATION

Determinations were made at 4-week intervals to discover what effects growing plants had on nitrification, nitrate nitrogen assimilation, and nitrogen fixation. In the nitrification studies 30 mgm. of ammonium sulfate were added to the equivalent of 100 gm. of air-dried soil and incubated 28 days. By averaging the results obtained in the nitrification studies for the various samplings from December 20 to April 11, inclusive, the increases in nitrate nitrogen were found to have been 15.38 mgm. in corn soils, 12.60 mgm. in kafir soils, and 13.43 mgm. in fallow soils. Average results from December 20 to March 14, inclusive, showed gains of 13.21 mgm. of nitrate nitrogen in wheat soils, 13.38 mgm. in barley soils, and 11.1 mgm. in fallow soils. These figures indicate a slightly increased nitrifying power in the corn, wheat, and barley soils and a decreased nitrifying power in kafir soils. Starkey (9) believed that 28 days is too long an incubation period and it is possible that differences that may have existed at an earlier period became obscured. The data obtained on the nitrate assimilating and nitrogen fixing powers of the soils likewise indicated that the growing plants had had but little effect on the nitrogen assimilating and nitrogen fixing powers of the organisms.

DISCUSSION

The addition of water to soil which had become more or less dry has been found by many investigators to result in increased biological activities. Such was the case in this experiment, as the numbers of microorganisms found in the soils increased with the addition of water and marked evolution of carbon dioxide resulted. The activities of the organisms were at their maximum by the third day and the effects of drying the soil were evident over a period of about four weeks, as was indicated by the evolution of carbon dioxide in the soils.

After the initial high period, larger amounts of carbon dioxide were found in the planted soils and the largest amounts found in any soil occurred when the plants were most active. Growth characteristics of the plants were reflected in distinct differences in the production of carbon dioxide. The largest amounts of carbon dioxide were produced when the plants had reached advanced stages of vegetative growth. After blooming and throughout the period of grain formation the evolution of carbon dioxide was pronounced.

What factors are involved in the increased carbon dioxide formation under plants is problematical. Lundegardh (4) believed that plant roots are responsible for only one-third of the increase and that the other two-thirds are due to microorganisms. Slightly increased numbers of bacteria and actinomycetes were found in the cropped soils in these experiments. Numbers of organisms, however, are not always indicative of the rates of decomposition of organic materials, as is seen in tables 4 and 5 where large numbers of microorganisms occurred in fallow soils throughout February, March, and April without any increase in the evolution of carbon dioxide. It seems logical to conclude that part of the increased production of carbon dioxide in cropped soils is due to biological activities. If Lundegardh's contention is true, the increased amounts of carbon dioxide in cropped soils are due, largely, to a greater physiological efficiency of the organisms rather than to increased numbers. This would indicate that plant roots excrete rather large amounts of soluble organic materials which may be oxidized readily by the organisms.

What effects increased production of carbon dioxide may have had in the soils growing kafir as compared to those growing corn, wheat, or barley are not clear. The kafir plants did not develop normally, but during their period of maximum vegetative growth larger amounts of carbon dioxide were produced in the soils than in wheat or barley soils at similar stages of growth of the plants. The amounts of carbon dioxide evolved in kafir soils were but slightly smaller than the amounts found in corn soils at similar stages of growth of the plants. Metzger (5) found that the concentration of bicarbonates in soils around the roots of corn plants was not much different than in the soil about the roots of kafir plants at similar stages of development. It appears unlikely that the solubility of soil nutrients is affected to a much greater extent by the production of carbon dioxide under corn than under kafir.

The data show that the greatest disappearance of soil nitrates took place before blossoming and at the time of grain formation. There is some correlation between the evolution of carbon dioxide and the disappearance of nitrates from the soil. There appears to have been little relationship between numbers of microorganisms and nitrates found in fallow soils at any one sampling. In general, increased numbers of organisms in fallow soils were accompanied by increased amounts of nitrates. If the organisms assimilated large amounts of nitrates the production of ammonia and nitrates evidently took place at a more rapid rate. Fluctuations in the amounts of nitrates found at different samplings indicate that considerable quantities of nitrates were

assimilated at times. The numbers and activities of the nitrifying organisms must have kept pace with the increase in the general microscopic population.

Starkey (9) found that growing corn and oats affected the nitrifying powers of a soil to only a slight extent. Sewell (7) compared the ammonifying and nitrifying powers of corn and kafir soils and found practically no differences in the effects of the two crops. Although there were indications in these experiments that the nitrifying powers of soils may have been slightly increased under corn, wheat, and barley and decreased under kafir the differences were too small to be significant. As nitrification and nitrate assimilation occur simultaneously in a soil and there is no way of determining the extent of either or the factors involved, conclusions from small differences in the data are not justifiable.

The method used in these investigations gave no indication of the presence of inorganic phosphorus in the soil solution. The soil solutions evidently contained phosphorus available to plants, but containing active calcium did not give tests for inorganic phosphorus. If the plants excreted organic compounds containing phosphorus the amounts were exceedingly small and the tests were not sufficiently delicate to show, with any degree of accuracy, what differences may have been caused by the growing plants.

Increased numbers of microorganisms in cropped soils are indicative of increased amounts of food materials. The tests used showed no differences in the amounts of water-soluble organic matter in the soils. No accumulation of easily decomposable materials under plants would be expected, however, except under sterile conditions.

SUMMARY

Determinations were made to ascertain the influences of the development of corn, kafir, wheat, and barley on numbers and certain activities of microörganisms in soils. Measurements were made periodically during the growth of the plants on carbon dioxide evolution; hydrogen-ion concentration of the soil solution; numbers of filamentous fungi, bacteria, and actinomycetes; nitrate content of the soils; nitrifying, nitrogen assimilating, and nitrogen fixing powers of the soils; and on the amounts of water-soluble phosphorus and water-soluble organic matter:

Wetting soils which had become more or less dry resulted in a marked evolution of carbon dioxide, the effects lasting about four weeks.

Larger amounts of carbon dioxide were produced in cropped than in uncropped soils, the largest amounts occurring when the plants were making their greatest vegetative growth.

The production of carbon dioxide was related to the growth characteristics of the plants and distinct for each of the plants.

Increased numbers of filamentous fungi were found under corn and decreased numbers under kafir, wheat, and barley. Larger numbers of bacteria and actinomycetes occurred in planted than in unplanted soils, the largest numbers being found under corn.

Increased numbers of microorganisms were not accompanied by increased evolution of carbon dioxide in fallow soils.

The plants made their largest draft on soil nitrates at the periods of maximum vegetative growth.

There were indications that the growing plants may have increased the nitrifying and nitrogen assimilating powers of the soils to a slight extent, but the differences were not significant.

Corn and kafir seem to decrease the hydrogen-ion concentration of the soil solution.

Although growing plants may produce changes in the quantities of water-soluble phosphorus and water-soluble organic matter, significant differences could not be detected by the methods used.

It appears likely that part of the increased numbers of microorganisms found under the growing plants were the result of the excretion of soluble organic matter by the growing plants.

The results obtained in these investigations indicate that conditions for microbial activities are somewhat more favorable under corn, wheat, or barley than under kafir.

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THE LONGEVITY OF LEGUME BACTERIA ON SEED, AS INFLU-
ENCED BY PLANT SAP[1]

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The importance of inoculating legumes with nitrogen fixing bacteria is universally recognized; and of the various methods practiced that of seed inoculation is the one most often utilized.   It is customary for the farmer to inoculate the seeds immediately prior to planting.   However, if bacteria can be made to survive on seedcoats for a sufficient length of time, then the seeds could be inoculated at some central point by the seed dealers, thus saving the planter much time and trouble.   Besides, inoculation could then be carried out in a more uniform and economical manner.

Some studies have been conducted as to the length of time the organisms will exist on seedcoats in the presence, and in the absence, of accessory substances.   Although plant juices have been utilized to grow bacteria in artificial media (3), yet no work has been reported concerning the utilization of plant sap as an aid to increase the viability and vitality of legume bacteria when these are dried on the seed.

HISTORICAL

Results obtained by observation indicate that the symbiotic nitrogen-gathering bacteria can exist for a number of years in soil, in the absence of the host plant.   Hopkins (6) states, ". . . . how long the bacteria will live in a soil without a leguminous crop upon which they feed is not definitely known.   Certainly they live for two or three years, but probably not more than five or six years."   According to Russell and Morrison (11) soybean nodule bacteria are capable of living under field conditions for a period of 17 years.   Albrecht (1) concludes that the legume bacteria of soybeans and red clover do not remain viable longer than six years in dry soil; whereas they will persist for a longer time in a soil left under natural conditions.

The conclusions arrived at concerning the longevity of *B. radicicola* when dried in the absence of soil are far different.   In 1905 (9), the U. S. Department of Agriculture distributed legume bacteria in a dried form, but these proved unsuccessful, as the organisms soon died on the dried strands of cotton.   In the same year, Kellerman and Beckwith (7) showed that a culture properly dried and protected from a moist atmosphere retains its vitality for 90 days; but, when dried under atmospheric conditions, the culture became sterile in 24 days.

Chester (4), in 1907, as a result of a review of the literature concerning the effect of desicca-

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tion on bacteria, mentions that; first, the resistance to drying is increased by the thickness of the layer; second, intermittent drying, such as occurs in air, results in rapid death of the organisms; third, organisms dried at body temperature die more quickly than when dried at lower temperatures; and fourth, old and weakened cultures are less resistant to drying than the highly virulent cultures. He dried *B. radicicola* at 35°C. (a temperature far above that to which the organism is usually exposed) and he states, *"Ps. radicicola* has little power to withstand drying . . . ."* As a result of his experiment with alfalfa seed, in which the seeds were sterile 18 days after drying, he says, *"Ps. radicicola* when dried on seed perish very quickly."

Temple (12), showed in 1916 that *B. radicicola* may live for months on the seed coat of cowpeas. Canada field peas were inoculated and, after drying, were stored in loosely stoppled bottles. At intervals of 30 days, seeds from this lot were planted, and nodules were found on the roots up to the fifth month of drying.

In 1919, results obtained by Fellers (5) were published. Soy-bean and alfalfa seeds, after being treated with inoculants for varying lengths of time, were carefully dried and stored in paper bags. At fixed intervals samples were tested for the presence of *B. radicicola* by means of the agar plate and by nodulation on plants grown from the seed. Organisms remained viable for at least six months, irrespective of the length of time the seeds were kept in contact with the inoculant. The greatest destruction of cells occurred during the first few hours; after this, they perished more slowly and uniformly. As gum tragacanth and similar substances do not aid greatly in keeping alive the bacteria on the seed, he does not recommend their use.

Richmond (10) inoculated seeds with muddy water prepare from soil, upon which the crop in question had been grown. He found that seeds so inoculated with muddy water from an acid soil were free of nodule bacteria within seven days, whereas seeds treated with muddy water prepared with neutral soil retained viable organisms for almost a year.

During the same year, 1926, Allicante (2) reported his results, concerning the viability of the nodule bacteria. He made a study of the effect of soil, glue, and sugar on the length of time the organisms will survive on the seed coat. Treatments containing sugar gave nodules superior in number and size to those treatments containing soil or glue. Sugar, according to this worker, protects against desiccation, because it possesses the characteristic of gathering moisture from the air, whereas, soil has a beneficial influence, because of the film of moisture surrounding the soil particles He found that seeds stored in cloth bags maintain the life of the legume organisms for a longer period of time than when stored in glass containers. These experiments, however, were terminated at the end of two months.

In 1927, Lockhead (8) reported experiments on alfalfa seeds inoculated with a suspension of organisms in sweetened milk, as compared with inoculated sand. Seeds treated by the "skim-milk" method retained viable bacteria on their seed coats for six months, and nodulation was favored by storing the seeds at low temperatures.

EXPERIMENTAL

Seeds of winter vetch and of sweet clover were used throughout this experiment.

Sterilization of seeds.—The seeds were placed in 1 to 500 solution of HgCl₂ for 5 minutes (5), removed and washed in four changes of sterile distilled water. When tested in bouillon tubes, 97 per cent of the seeds proved to be sterile, although no reduction in their germination was noticeable. Tests for mercury showed no trace of this element.

Medium used for Petri dishes.—A modified Ashby's medium was used for growing the organisms and for counting them (5). One liter of the medium contained 10 gm. mannite, 0.2 gm. KH_2PO_4, 0.2 gm. $MgSO_4$, 0.1 gm. $CaSO_4$, 1.0 gm. $CaCO_3$, and 18 gm. agar.

Preparation of the inoculum.—Twenty-five grams of washed sweet clover and winter vetch nodules were separately crushed in 500 cc. of sterile water, after which the infusions were filtered through several layers of cheesecloth. Then 0.1-cc portions were mixed with the Ashby's medium in 250-cc. Erlenmeyer flasks, and inoculated at 28°C. for 10 days. The surface colonies were removed by a minimum amount of sterile water by means of shaking and by rubbing with a sterile spatula. Like strains of the organisms were then mixed together in a sterile flask, and about 20 cc. of 25 per cent sterile sugar solution was added to each of the two flask. The contents of each flask were then divided into nine portions. The liquid in three flasks of each strain was diluted with 10 cc. of sterile water; to another set of three flasks, 10 cc. of sterile milk was added; while in the third set of three flasks, 10 cc. of plant sap was used.

Preparation of the plant sap.—The plant sap was obtained by compressing the plant and extracting the liquid. The plants were young and succulent, the clover being about 1 month old, and the vetch 3 weeks. The expressed juices were sterilized before being added to the inoculum.

Reaction of the inoculum.—The reaction of each inoculum was adjusted by means of dilute HCl and NaOH to pH 6.5, 7.0, 7.5, and verified by means of the potentiometer.

Inoculation of the seeds.—After the seeds had been sterilized and dried between sterile blotting paper, about 50 gm. of clover seed was put into each of the flasks containing the clover organism, and about 80 gm. of vetch seed was placed in the flasks containing the vetch organism. These were allowed to remain 30 minutes in the inoculum, then they were placed between sterile blotting paper and dried.

Storage of inoculated seeds.—Each treatment of the dried inoculated seeds was divided into two portions and placed in sterile cloth bags. One set of these bags was stored in the laboratory, while the other set was stored in a refrigerator at 5°C.

Bacteriological technique.—Fifteen vetch seeds and 200 clover seeds were removed from each of the bags by means of sterile forceps, placed in flasks containing 100 cc. sterile water, and allowed to stand for 60 minutes. These were then shaken for 5 minutes, dilutions made, and then plated on the Ashby's medium (5). The dilutions commonly used were: 1/1,000, 1/100,000 and 1/1,000,000. Each dilution was poured in duplicate and the plates were incubated at 27°–30°C. for 10 days, after which they were counted.

Greenhouse tests.—Two-gallon earthenware pots, containing white quartz sand, were used for the nodulation tests. Each test was run in duplicate. Each of the pots planted to vetch had 15 seeds, while those planted to clover had 200 seeds. After 40 days the sand and plants were removed and the nodules counted.

The plants were watered with a nitrogen-free solution containing the following salts per liter: 10 gm. $CaSO_4$, 4.6 gm. KH_2PO_4, 2.3 gm. $MgSO_4$, and 0.25 gm. $FeSO_4$.

TABLE 1

The longevity of B. radicicola per 15 winter vetch seeds, and nodules per plant

WEEKS OF STORAGE	INOCULUM ALONE						INOCULUM PLUS PLANT SAP						INOCULUM PLUS MILK					
	pH 6.5		pH 7.0		pH 7.5		pH 6.5		pH 7.0		pH 7.5		pH 6.5		pH 7.0		pH 7.5	
	Colonies ×1,000	Nodules	Colonies ×1,000	Nodules	Colonies ×1,000	Nodules	Colonies ×1,000	Nodules	Colonies ×1,000	Nodules	Colonies ×1,000	Nodules	Colonies ×1,000	Nodules	Colonies ×1,000	Nodules	Colonies ×1,000	Nodules
Seeds stored at room temperature																		
0	690	4 3	1,295	18 2	700	14 3	1 625	12 7	490	8 9	1,650	9 0	400	10 4	700	15 3	690	20 0
1	150	2 4	90	6 3	100	12 5	1,190	10 9	750	10 0	790	10 9	260	10 4	340	11 0	160	10 5
3	85	0 4	150	8 6	40	0 2	650	4 6	200	16 0	710	18 4	111	8 9	120	10 3	160	12 1
6	10	2 0	25	3 6	50	1 2	500	4 5	600	8 0	625	3 2	110	2 1	90	0 7	5	1 9
12	15	1 9	25	0	14	4 9	90	4 9	95	2 2	250	5 4	0	0 2	10	0	4	1 0
24	2	0	0	0	10	0	2	5 2	100	10 2	5	0	250	1 2	14	0	4	3 2
56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Seeds stored at 5°C																		
1	100	15 9	60	18 2	100	5 4	1 750	11 1	690	10 4	1,200	9 7	175	7 6	125	2 5	100	0 7
3	75	6 4	100	7 2	150	3 3	1,300	12 2	1,300	25 4	750	19 9	100	0 5	90	4 6	100	9 5
6	15	0 2	90	1 6	100	1 1	400	8 2	790	10 4	700	2 5	100	6 3	490	1 6	1,120	1 0
12	4	0 2	10	1 0	60	0 9	120	3 9	360	7 5	60	10 2	100	0	20	0 2	40	0 6
24	4	0	15	1 2	20	2 0	100	2 6	350	7 4	100	1 0	0	0	50	0 2	40	0 9
56	0 1	0	0 9	0	0	0	0 3	0	1 1	0	0 2	0	0 2	0	0	0	0	0

TABLE 2

The longevity of B radicicola per 200 sweet clover seeds, and nodules per plant

Seeds stored at room temperature

WEEKS OF STORAGE	INOCULUM ALONE						INOCULUM PLUS PLANT SAP						INOCULUM PLUS MILK					
	pH 6 5		pH 7 0		pH 7 5		pH 6 5		pH 7 0		pH 7 5		pH 6 5		pH 7 0		pH 7 5	
	Colonies ×1,000	Nodules	Colonies ×1,000	Nodules	Colonies ×1 000	Nodules	Colonies ×1,000	Nodules	Colonies ×1,000	Nodules	Colonies ×1,000	Nodules	Colonies ×1,000	Nodules	Colonies ×1,000	Nodules	Colonies ×1,000	Nodules
0	560	2 4	1,275	1 1	3,162	6 9	900	12 9	1,425	10 0	3,690	9 4	280	11 1	590	15 9	160	16 2
1	265	6 3	110	1	150	7 3	725	10 0	925	30 2	430	20 1	100	4 0	350	2 7	160	7 6
3	212	0 3	75	1 0	110	0 5	150	10 0	375	12 0	100	4 0	214	4 4	110	5 0	50	3 1
6	6	0 1	15	3 2	20	1 2	190	2 5	208	4 1	122	5 2	175	0 2	100	1 0	110	3 2
12	1	0 0	4	0 0	0	1 2	15	0 4	4	8 1	8	2 0	16	0	45	0 8	120	1 3
24	1	1 9	0	0 0	2	0 4	25	4 8	100	1 2	90	1 5	0	0 4	260	0 0	500	1 0
56	0	0 0	0	0 0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Seeds stored at 5°C.

WEEKS OF STORAGE	INOCULUM ALONE						INOCULUM PLUS PLANT SAP						INOCULUM PLUS MILK					
	pH 6 5		pH 7 0		pH 7 5		pH 6 5		pH 7 0		pH 7 5		pH 6 5		pH 7 0		pH 7 5	
	Colonies ×1,000	Nodules	Colonies ×1,000	Nodules	Colonies ×1 000	Nodules	Colonies ×1,000	Nodules	Colonies ×1,000	Nodules	Colonies ×1,000	Nodules	Colonies ×1,000	Nodules	Colonies ×1,000	Nodules	Colonies ×1,000	Nodules
1	350	2 6	285	3 8	590	9 4	1,125	10 2	2,200	15 8	1,100	7 4	125	8 7	130	0 4	110	10 5
3	230	0	345	3 2	115	6 2	750	15 0	1,600	8 0	275	10 0	140	0 3	250	0 8	125	2 0
6	50	0 2	120	5 6	30	2 4	390	4 5	750	6 4	1,360	3 9	100	1 0	125	1 8	30	4 5
12	10	0 9	78	1 0	105	1 2	340	8 9	1,000	2 2	942	4 4	48	0 4	60	0	40	2 3
24	5	0 5	70	0 8	90	1 0	250	7 2	40	6 0	195	1 5	60	0 6	60	2 6	75	0
56	0	0	0	0	0	0	2	0	1 7	0	0 1	0	0	0	0 3	0	0 1	0

Immediately after the seeds were inoculated and dried, they were tested for the number of viable organisms, and another set was planted in the pots for nodule counts.

The seeds were then stored in the manner indicated, and tests for bacteria and for nodule counts were made at the end of 1, 3, 6, 12, 24, 56, and 78 weeks. The results are presented in tables 1 and 2.

The number of nodules per plant is calculated from the seeds that germinated and grew.

DISCUSSION

The data presented in the tables indicate that the addition of plant sap to the inoculum assures the preservation of a larger number of viable organisms on the seed than is possible with the use of milk or water. Although, at the end of 24 weeks, positive results were obtained from almost all the treatments, yet it is obvious that the inoculum plus plant sap gave the greatest number of bacteria and also the highest degree of nodulation. At the 56-week period, no nodule bacteria could be found on the seeds stored at room temperature; while those seeds treated with plant sap or with milk, and placed at 5°C. showed a few of the organisms. The former gave slightly higher results, but no nodules could be found on the plants grown from these seeds. As only certain cells of *Bacillus radicicola* have the power to penetrate the root and produce nodules, there must be a minimum number of these organisms present on the seed, for Thornton and Ganzulee (13) have shown that the degree of nodulation is closely associated with a predominance of the coccoid or motile form of *B. radicicola*. The increased nodulation which occurs with the addition of plant sap for shorter storage periods may be caused by the greater activation of these coccoid forms.

The differences in the reaction of the inoculum are so slight that nothing definite can be concluded; yet it appears that pH 7 gives the best result. Wilson (14), noting that ageing of seed is accompanied by a change in reaction, as a result of respiration, believes this increase in acidity may have some bearing on the longevity of legume bacteria on the seed.

If the rate of mortality due to desiccation is constant (5), then nodulation could be expected after the 24-week period, especially in the seeds treated with plant sap. However, the inoculation of the seed, and its subsequent storage, must be carefully controlled in order to assure a fair degree of nodulation after a period of time.

SUMMARY

Vetch and clover seeds were sterilized and kept for 30 minutes in contact with their specific inoculum, after which they were dried and stored, some at room temperature, and the remainder at 5°C.

The organisms on seeds inoculated with inoculum containing plant sap from

their specific plants withstood desiccation to a higher degree than organisms on seeds similarly treated with inoculum containing milk.

The slight differences in the reaction, pH 6.5, 7,7.5, as used in this experiment had but little effect on the viability of the legume bacteria.

Refrigeration of seeds inoculated with *B. radicicola* increased the longevity of the organisms attached to the seed coat.

Although seeds treated with plant sap retained viable bacteria in fairly large numbers until the twenty-fourth week, yet such storage for seeds so inoculated is not recommended at this time.

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